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FIREFIGHTERS AND ACUTE MYOCARDIAL INFARCTION: UNDERSTANDING MECHANISMS AND REDUCING RISK

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For Luca

And the firefighters worldwide who work tirelessly to protect our communities

Abstract

Acute myocardial infarction is the commonest cause of death in firefighters, accounting for 45% of all deaths on duty. Compared with an average life expectancy of 77 years in the general population, the average age of cardiovascular death in firefighters is 50 years suggesting that occupational hazards are responsible for premature disease. The risk of acute myocardial infarction is increased 12- to 136-fold during rescue and firefighting duties, and is likely to reflect a combination of factors including strenuous physical exertion, mental stress, heat and pollutant exposure. Previous studies have established that the duties of a firefighter, in particular fire suppression, put inordinate strain on the cardiovascular system yet the exact mechanisms underlying the increased risk of myocardial infarction remain poorly defined.

In a series of studies, I assessed the effect of occupation-specific risk factors on cardiovascular health in a combination of controlled and real-life studies in order to better define these mechanisms, hypothesising that exposure to high temperatures, strenuous physical exertion, psychological stress and air pollution either alone or in combination caused vascular dysfunction and thrombosis.

In order to assess if firefighters had a greater cumulative risk of cardiovascular disease due to their occupation at baseline, I assessed the

cardiovascular function of group of healthy, off-duty firefighters and compared this to a group of healthy age- and sex-matched off-duty police officers; an occupational group with similar responsibilities but a much lower risk of on-duty cardiovascular events. I was able to demonstrate that traditional cardiovascular risk factors, vascular endothelial function and thrombogenicity were similar in the two groups concluding that the excess of cardiovascular events and deaths in on-duty firefighters are due to the acute and transient effects of strenuous physical exertion, psychological stress, heat and exposure to air pollutants.

Having established that off-duty firefighters had no apparent increased risk of cardiovascular events, I then went on to clarify the effects of combustion derived air pollution in the form of wood smoke on the cardiovascular system. The suppression of wildland or forest fires is globally the single most important duty of the fire service. Previous work within our institution has demonstrated the adverse effects of combustion derived air pollution, in the form of diesel exhaust, on the cardiovascular system. In a similar fashion, I assessed the effect of a wood smoke inhalation in a group of healthy off-duty firefighters by performing controlled exposures to wood smoke utilising a unique and well characterised facility. Interestingly, unlike diesel-exhaust, the exposure to wood smoke had no adverse effect on vascular endothelial function or thrombogenicity in this group concluding that cardiovascular events during wildland fire suppression may not be directly related to wood

smoke inhalation but instead precipitated by other mechanisms such as strenuous physical exertion or dehydration.

Latterly, I proceeded to evaluate the effects of strenuous physical exertion and heat exposure by comprehensively assessing a number of cardiovascular end points following controlled exposure to a fire simulation activity in a group of healthy, off-duty firefighters. I was able to demonstrate that exposure to extreme heat and physical exertion impaired vasomotor function and increased thrombus formation. Moreover, I demonstrated cardiac troponin concentrations increased suggesting that fire suppression activity may cause myocardial injury. These important findings suggest pathogenic mechanisms to explain the association between fire suppression activity and acute myocardial infarction.

In the final phase of work, I endeavoured to assess the effects of real-life firefighter activities on the cardiovascular system. In an ambitious study, I attempted to undertake a comprehensive assessment of cardiovascular function in healthy firefighters following three periods of duty: fire suppression, alarm response and non-emergency activity. I was unable to complete enough studies to adequately power an analysis and draw any firm conclusions about the effect of these duties on cardiovascular health. Further work is required in a real-world setting to more clearly define the

occupational risk factors underlying the increased risk of cardiovascular events associated with specific firefighter duties

Understanding the biological mechanisms and environmental factors that predispose firefighters to cardiovascular events is essential if we are to develop effective methods for the prevention of acute myocardial infarction on-duty. This body of work has greatly improved the understanding of the mechanisms underlying the increased risk of cardiovascular events on duty and calls for the immediate evaluation of current practice in order to minimise risk to firefighters in the future. Examples of where improvements should be made include strategies to ensure adequate hydration and cooling following exposure to heat and physical exertion, change to working patterns to limit the duration of extreme exposures, and education, training and screening programmes to reduce the impact of traditional and occupational cardiovascular risk factors.

Lay Summary

Heart attacks are the commonest cause of death in firefighters, accounting for 45% of deaths at work. Compared with an average life expectancy of 77 years in the general population, the average age of death from heart disease in firefighters is 50 years suggesting that job-related hazards are responsible for premature disease. The risk of heart attacks is increased by 12- to 136-times during rescue and firefighting duties and is likely to reflect a combination of factors including strenuous physical exertion, mental stress, heat and air pollution exposure. Previous studies have established that the duties of a firefighter, in particular tackling and extinguishing fires, puts a lot of strain on the heart and blood vessels yet exactly why heart attacks occur in this group remains poorly understood.

In a series of studies, I assessed the effect of risk factors related to a firefighter's job on the heart in blood vessels in a combination of controlled and real-life studies in order to better understand why heart attacks occur, assuming that exposure to high temperatures, strenuous physical exertion, mental stress and air pollution either alone or in combination cause dysfunction of the heart and blood vessels.

In order to assess if firefighters have a greater risk of heart disease due to their job whilst off-duty, I assessed the heart and blood vessel function and

blood clot formation in a group of healthy, off-duty firefighters and compared them to a group of off-duty police officers of the same age and sex. Police officers are a group with similar job responsibilities but a much lower risk of on-duty cardiovascular events. I was able to show that usual heart disease risk factors such as high blood pressure and smoking, blood vessel function and blood clot formation were similar in the two groups concluding that the excess number of heart attacks and deaths due to the same in on-duty firefighters are due to the sudden effect of strenuous physical exertion, mental stress, heat and exposure to air pollutants.

Having established that off-duty firefighters had no apparent increased risk heart attacks, I then went on to clarify the effects of air pollution, in the form of wood smoke, on the cardiovascular system. Firefighters are exposed to high levels of air pollution in the form of wood smoke when fighting wildland or forest fires. Previous work within our research group has demonstrated that air pollution, in the form of diesel exhaust, is extremely bad for the heart and blood vessels. In a similar fashion, I assessed the effect of wood smoke inhalation in a group of healthy off-duty firefighters by performing controlled exposures to wood smoke using a unique exposure facility. Interestingly, unlike diesel-exhaust, the exposure to wood smoke had no adverse effect on blood vessel function or blood clotting in this group concluding that heart attacks during wildland firefighting may not be directly related to wood smoke inhalation but instead caused by strenuous physical exertion or dehydration.

I then went on to evaluate the effects of strenuous physical exertion and heat exposure by comprehensively assessing the effects on the heart and blood vessels following a controlled exposure to a fire simulation activity in a of a group of healthy, off-duty firefighters. I was able to show that exposure to extreme heat and physical exertion impaired blood vessel function and increased blood clotting. I also showed there was an increase in a protein called troponin which is released by the heart muscle in response to lack of oxygen suggesting that fire simulation activity may cause damage to the heart muscle. These important findings begin to explain why firefighters suffer heart attacks around the time of tackling and extinguishing fires.

In the final phase of work, I attempted to assess the effects of real-life firefighter activities on the heart and blood vessels. In an ambitious study, I attempted to undertake a comprehensive assessment of heart and blood vessel function in healthy firefighters following three periods of duty: tackling and extinguishing a fire, responding to an alarm and a non-emergency activity.

Understanding the factors that put firefighters at risk of heart attacks is essential if we are to develop effective methods to prevent them occurring. This body of work has greatly improved the understanding of why firefighters have an increased risk of heart attacks on duty and calls for the immediate

evaluation of current practice in order to minimise risk to firefighters in the future. Examples of where improvements could be made include strategies to ensure adequate hydration and cooling following exposure to heat and physical exertion, change to working patterns to limit the duration of extreme exposures, and education, training or screening programmes to reduce the impact of usual and job-related risk factors for heart disease.

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DECLARATION

This thesis represents research undertaken in the Centre for Cardiovascular Science, University of Edinburgh, Royal Infirmary of Edinburgh and the Department of Respiratory and Allergy Medicine, Umeå University, Sweden during the period of 2011 and 2014 whilst working as a clinical research fellow.

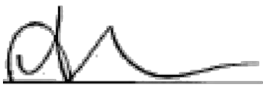
The British Heart Foundation Project Grant (PG/11/27/28842) sponsored these studies and supported my salary. The Colt Foundation contributed towards my university PhD fees from 2011 – 2015. The wood smoke exposure study was supported by additional funding from the Swedish Heart and Lung Foundation.

I have composed this thesis myself. I conceived the research studies together with my primary supervisor Professor Nicholas Mills but otherwise I personally initiated, conducted and performed the data analysis of all the studies presented in this thesis. In keeping with the nature of collaborative research, assistance with some of the studies in Sweden was gratefully received from my colleague Dr Jon Unosson at Umea University. Assistance was also provided in the technical supervision of the exposures and laboratory assays as acknowledged.

Excerpts of Chapter 1 have been published in peer-reviewed journals and in a book chapter. Chapters 3 and 4 have been published in peer-reviewed journals. Appropriate copyright permission has been sought for inclusion of the printed journal manuscripts.

The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged.

All studies were undertaken in accordance with the regulations of the local Ethics Board within NHS Lothian and Umeå University Hospital and with the Declaration of Helsinki of the World Medical Association.



AMANDA L HUNTER 1st March 2018

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The project was designed in response to questions raised by firefighters and occupational physicians from the Lothian, Borders and Fife Fire and Rescue Services, following the death of two young Scottish firefighters from acute myocardial infarction in 2008. Their ultimate sacrifice highlighted the need for a better understanding and record of the causes of death amongst on-duty firefighters. I would like to pay a special thanks to those whose advice and knowledge proved invaluable in designing this project: professional firefighters within the Scottish Fire and Rescue Service (previously Lothian and Borders), the Deputy Chief Fire Officer, the Chairman of the Health and Welfare Group of the Chief Fire Officers Association (Scotland) and representatives of the Fire Brigades Union. I would also like to thank Dr James Marshall (Consultant Occupational Health Physician), formerly of

Lothian and Borders Fire and Rescue Service and Dr Richard Graveling (Consultant Occupational Health Physician) at the Institute of Occupational Medicine for their valuable input and advice. To the staff at the Scottish International Fire Training Centre for their assistance, patience and ingenuity, thank you.

I would like to acknowledge the British Heart Foundation for supporting these studies through the Project Grant (PG/11/27/28842). Without their generosity, this thesis and the research material presented would not have been possible. I would also like to thank the Colt Foundation and especially Jackie Douglas for agreeing to fund the PhD University fees. Their financial and professional support has been invaluable.

I was fortunate to have the opportunity to travel to and spend over 2 months in Umeå, Sweden during this period of research. It was a great honour to work within an established collaboration. The hospitality and friendship I was shown was second to none. My colleagues and collaborators, Anders Blomberg and Thomas Sandström were fantastic but I would especially like to thank good friends Jenny Bosson, Jon Unosson and Greg Rankin for all their help, support and fabulous company; Jenny in particular for her Michelin Star-quality cooking. To Frida Holmstrom, Annika Johansson and Jamshid Pourazar, a big thanks for their incredible help and support with the studies.

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I am forever indebted to the participants of these research studies who generously gave up their time to participate in these studies. They did so in the hope that they might improve their working environment for themselves and others in order to prevent heart disease amongst firefighters in future. They can be proud of their contribution.

Finally, a huge debt of gratitude is owed to my family. My mum and dad, Lynn and Gordon, have supported me through everything I have achieved in life and all of those achievements are theirs in part. I thank them especially for helping take care of Luca to let me write this thesis. Who could have predicted that maternity leave would not have provided endless weeks of free time to write a thesis? Not me. To my husband Chris, thank you for putting up with me. I love you all and thank you for all your love and support.

ABBREVIATIONS

ACH – acetylcholine

aPTT - activated partial thromboplastin time

ANOVA – analysis of variance

APC - allophycocyanin

BBC – British Broadcasting Corporation

BK - bradykinin

BMI – body mass index

bpm – beats per minute

CAD – coronary artery disease

CI – confidence intervals

CO – carbon monoxide

CO_2 – carbon dioxide

CRP – C-reactive protein

CT – computed tomography

CTCA – computed tomography coronary angiography

CV – coefficient of variation

ECG – electrocardiogram

EDTA - ethylene diamine tetra-acetic acid

ELISA – enzyme-linked immunosorbent assay

FBF – forearm blood flow

FITC - fluorescein isothiocyanate

h - hours

HDL – high density lipoprotein

LDL – low density lipoprotein

min - minutes

MI – myocardial infarction

METs – metabolic equivalents

NO – nitric oxide

NO_2 – nitrogen dioxide

NO_x – nitrogen oxides

PAH – polyaromatic hydrocarbon

PAI-1 – plasminogen activator inhibitor-1

PE - phycoerythrin

PM – particulate matter

PM_1 – particulate matter less than 1μm in diameter

$PM_{2.5}$ – particulate matter less than 2.5μm in diameter

PM_{10} – particulate matter less than 10μm in diameter

PPACK - D-Phenylalanine-L-propyl-L-arginine chloromethyl ketone

PPE – personal protective equipment

SCBA – self-contained breathing apparatus

SCD – sudden cardiac death

SD – standard deviation

SEM – standard error of the mean

SNP – sodium nitroprusside

SO_2 – sulphur dioxide

t-PA – tissue plasminogen activator

UK – United Kingdom

US – United States of America

$VO_{2\ Max}$ – maximal rate of oxygen consumption measured during exercise

VP – verapamil

vWF – von Willebrand factor

WHO – World Health Organisation

°C – degrees Celsius

CHAPTER ONE

INTRODUCTION

Extracts from this chapter have been published in:

Hunter AL, Mills NL & Newby DE. Combustion-derived air pollution and cardiovascular disease. *British Journal of Hospital Medicine.* 2012;73(9):492–7.

Donaldson K, **Hunter AL**, Poland C & Smith S. Mechanisms of Action of Combustion-derived Nanoparticles. In: *Toxicology, Survival and Health Hazards of Combustion Products*. Edited by Purser DA, Maynard RL & Wakefield JC. Royal Society of Chemistry. 2015

Chapter 1: INTRODUCTION

1.1 OVERVIEW

Firefighters have long held the mantle of society's most respected and revered profession, risking their own lives to save the lives of others. The risks of entering burning buildings or attempting to free a passenger from the wreckage of a vehicle at a busy roadside are inherent. When firefighters enter the profession as young adults they do so under no illusion that these risks exist. The technological advances in recent years have afforded the highest specification firefighter protective equipment (i.e. breathing apparatus and personal protective equipment) yet the number of on-duty firefighter deaths remains the same.

The commonest cause of on-duty firefighter death is cardiovascular disease, and despite the steady decline in the average number of deaths among firefighters over the last decade, cardiovascular events still accounted for 42 % of all on-duty deaths in the last 5 years (Malik and Widlansky, 2015), about 90 % of which are due to coronary artery disease (Kales et al., 2007). With approximately 1.2 million operational firefighters in the United States (US) encompassing 345,600 career and 814,850 volunteer firefighters, this translates to 45-50 cardiovascular deaths annually with a further 765-850 firefighters having a non-fatal cardiovascular event (Karter MJ, 2011). In the United Kingdom (UK), on duty firefighter cardiovascular events and deaths

are not recorded, nationally collated or reported as fastidiously as they are in the US and therefore the exact numbers of firefighters who have died from or survived a cardiovascular event in the UK are currently unknown.

Collectively firefighters are more likely to die of a cardiovascular event on-duty than any other worker. In comparison with other professions with similar responsibilities i.e. emergency call-outs, physical exertion and dangerous duties, on-duty firefighters have an abnormally high incidence of cardiovascular death. As aforesaid cardiovascular events account for 45 % of deaths amongst on-duty firefighters contrasting with 22 % of deaths in on-duty police officers, 11 % of on-call emergency medical service workers and 15 % of all deaths at work. Where firefighters differ from other emergency professionals, and indeed from other workers in general, is the exposure to strenuous physical activity, emotional stress, intense heat and environmental pollutants, which can be encountered in isolation in some of their professional duties or collectively when fighting a fire.

Together such occupational exposures place inordinate strain on the cardiovascular system and represent the final step in a pathophysiological process leading to cardiovascular events in susceptible or vulnerable individuals. (Smith et al., 2013, Soteriades et al., 2011). Evidence that coronary events are triggered by specific firefighting duties first became apparent when it was demonstrated that the circadian pattern of deaths

paralleled the pattern of emergency response dispatches and were more frequent later in the day (Kales et al., 2003) unlike in the general population where cardiovascular events occur more frequently between 6 and 9 a.m. (Tofler et al., 1987, Muller et al., 1985). Fire suppression duties specifically and markedly increase the risk of suffering or dying from an acute coronary event or arrhythmia with a 12- to 136-fold greater risk as compared with non-emergency duties (Kales et al., 2007). This is despite the fact that fire suppression duties only comprise 1-5 % of a firefighter's professional time. However, there is an increase in the risk of cardiovascular death associated with other specific emergency duties as compared to non-emergency duties such as alarm response (odds ratio 2.8-14.1), alarm return (odds ratio 2.2-10.5) and physical exertion (odds ratio 2.9-6-6). This suggests there are multiple occupational-related risk factors or triggers at play.

Studies that have examined autopsy findings of firefighters who have suffered sudden cardiac death (SCD) have found that the majority of firefighters have had evidence of coronary atherosclerosis (Kales et al., 2003, Geibe et al., 2008). The absence of coronary thrombus or evidence of plaque rupture in some autopsies of firefighters succumbing to on-duty sudden cardiac death suggests that arrhythmias may also account for fatalities (Geibe et al., 2008, Yang et al., 2013). In the absence of occlusive thrombus, the same occupational exposures may have lowered the threshold

for an arrhythmia by increasing cardiac sympathetic activation leading to SCD.

Whilst we are acutely aware of the association between firefighting and cardiovascular events, the mechanisms behind this increased risk have not yet been clearly elucidated.

1.2 PRE-EXISTING RISK FACTORS FOR CARDIOVASCULAR DISEASE

As a group, firefighters should be healthier than the general population as they are highly selected at recruitment, required to meet pre-specified fitness and health standards, creating the so-called “healthy-worker effect”. Indeed, most studies that have compared overall cardiovascular mortality of firefighters with the general public have demonstrated that firefighters do not have an increased risk of cardiovascular death overall (Smith, 2011). However, when on-duty, firefighters have one of the highest occupational risks of cardiovascular events. Previous work has demonstrated that firefighters who have died from or survived cardiovascular events have a higher prevalence of traditional cardiovascular risk factors when compared to healthy controls (Geibe et al., 2008, Holder et al., 2006). Cardiovascular health is largely determined by fitness and the presence of risk factors for cardiovascular disease. It is therefore highly plausible that there are a

susceptible few firefighters with established cardiovascular risk factors in whom cardiovascular events occur.

1.2.1 FIREFIGHTER FITNESS

Firefighting is one of the most physically demanding occupations. Poor physical fitness and health not only places firefighters at high personal risk when performing occupational duties, but also the firefighting team and the public as a whole. Successful completion of activities performed by firefighters requires high levels of aerobic and anaerobic fitness, muscular strength and endurance. Firefighters are 'expected' to have above average fitness levels which they are required to maintain for the duration of their career. Currently under review within the fire service in the UK is the recommended minimum aerobic capacity for safe performance as a firefighter. Indeed, if we are to consider the usual deterioration of cardiopulmonary fitness with age (***Table 1.1*** and ***1.2***), a decline in $VO_{2\ Max}$ of 5-10 % per decade (Fleg, 2005), then at inception of a firefighters career, he/she should be required to have supra-maximal fitness levels when compared to age- and sex-matched populations. Furthermore, maintenance of such fitness levels is challenging with advancing age and especially so for females who will persistently require fitness levels above the 80th centile for age and well above the 90th centile in their latter working years (***Table 1.2***). UK firefighters have cardiopulmonary fitness level assessments triennially or

more frequently should they not attain the recommended minimal acceptable $VO_{2\ Max}$. By contrast, in the US, most fire departments require firefighters to attain a certain level at entry, but few departments require incumbent firefighters to maintain these standards. Inactive firefighters have a 90 % greater risk of myocardial infarction than those who are aerobically fit (Peate et al., 2002).

Table 1-1 Peak oxygen consumption (ml/kg/min) in male non-athletes

Percentile	Age (years)				
	20-29	**30-39**	**40-49**	**50-59**	**>60**
90	55.1	52.1	50.6	49.0	44.2
80	52.1	50.6	49.0	44.2	41.0
70	49.0	47.4	45.8	41.0	37.8
60	47.4	44.2	44.2	39.4	36.2
50	44.2	42.6	41.0	37.8	34.6
40	42.6	41.0	39.4	36.2	33.0
30	41.0	39.4	36.2	34.2	31.4
20	37.8	36.2	34.6	31.4	28.3
10	34.6	33.0	31.4	29.9	26.7

Reference $VO_{2\,Max}$ by percentiles according to American College of Sports Medicine

Table 1-2 Peak oxygen consumption (ml/kg/min) in female non-athletes

Percentile	Age (years)				
	20-29	**30-39**	**40-49**	**50-59**	**>60**
90	49.0	45.8	42.6	37.8	34.6
80	44.2	41.0	39.4	34.6	33.0
70	41.0	39.4	36.2	33.0	31.4
60	39.4	36.2	34.6	31.4	28.3
50	37.8	34.6	33.0	29.9	26.7
40	36.2	33.0	31.4	28.3	25.1
30	33.0	31.4	29.9	26.7	23.5
20	31.4	29.9	28.3	25.1	21.9
10	28.3	26.7	25.1	21.9	20.3

Reference $VO_{2\,Max}$ by percentiles according to American College of Sports Medicine

It is widely acknowledged that different firefighting duties require different levels of energy expenditures, yet several studies and expert consensus conclude that fire suppression, although performed relatively infrequently, is a strenuous, arduous task requiring a minimum oxygen uptake of 42 mL/kg/min or 12 metabolic equivalents (METs) to perform the task safely (Smith et al., 2013, Baker et al., 2000, Davis and Dotson, 1987). Sothmann et al previously assessed the ability of firefighters to complete a fire suppression task based on their $VO_{2\ Max}$ (Sothmann et al., 1991). Those with a $VO_{2\ Max}$ >41 mL/kg/min successfully completed the task lasting 9 minutes. 20% of those with a $VO_{2\ Max}$ between 33.5-41 mL/kg/min and 50 % of those below 33.5 ml/kg/min failed to complete the task and thereby concluded that 33.5 mL/kg/min should be the cut off point for safe level of cardiopulmonary fitness as those working at or below this level would have little in reserve while working in an emergency situation (Mark et al., 1990). Others have previously recommended 45 mL/kg/min as the safe limit (Gledhill and Jamnik, 1992). The current entry requirement for recruitment to the UK Fire Service is a minimum $VO_{2\ Max}$ of 42 mL/kg/min regardless of age. Despite this initial capability testing at entry, there is no national policy or official accepted guidance regarding the implementation of minimum physical fitness standards or the maintenance of fitness thereafter. Nowadays, firefighters use the most modern and technically advanced protective equipment, a recent UK study set up to evaluate a cardiorespiratory fitness standard for operational firefighters demonstrated that a $VO_{2\ Max}$ of 42.3mL/kg/min is

necessary to perform the most arduous firefighting tasks safely and effectively (Siddall, 2014). Following the publication of this study, the British Broadcasting Corporation (BBC), as part of an investigative series looking into firefighter fitness levels, obtained data demonstrating that 2,890 of 24,272 firefighters had a $VO_{2\ Max}$ measured below 42 mL/kg/min (data from 36 of fire services in the UK). 665 firefighters failed to reach a lower standard of 35 mL/kg/min, below which firefighters are at risk of sudden cardiac death particularly whilst undergoing high levels of physical exertion and should be removed from active duty until they attain a suitable standard for remedial training.

Modern protective personal equipment (PPE), whilst offering superior protection, comes at a cost of increased cardiac workload. The heavy weight, bulkiness and stiffness of the kit restricts normal movement and the increased thermal insulation from PPE and self-contained breathing apparatus (SCBA) results in the premature onset of muscle fatigue and impaired thermoregulation (elevation of core body temperature) which consequently leads to a reduction in work capability and duration. Exercise time on a treadmill in full firefighting kit is shorter than unimpeded exercise without. Maximal performance time was reduced by approximately 7 min (35 %) during uphill walking and 11 min (52 %) during treadmill running without an incline when wearing full PPE and SCBA. In this study participants were also tested with running shoes and with rubber boots (Lee et al., 2013).

The added weight of the boots further decreased exercise duration. Studies have also demonstrated a 10-17 % reduction in $VO_{2\,Max}$ when wearing SCBA alone (Louhevaara, 1984, Louhevaara et al., 1986) and 17-20 % with PPE and SCBA (Dreger et al., 2006, Petruzzello et al., 2009, Raven et al., 1977) during exercise. This indicates a greater metabolic burden on the wearer imposed by the PPE. The energy cost of wearing the PPE is increased by 11-20 % (Baker et al., 2000b). Furthermore, the adverse impact of PPE on exercise duration, hydration status and thermoregulation is likely to be greater in individuals with obesity and other medical problems. Smokers have also been shown to have reduced exercise duration when compared to non-smokers in PPE and SCBA (Goto et al., 2004, Raven et al., 1977).

A firefighter's perception of fitness level can be impaired with no association between perceived fitness levels and actual levels of aerobic capacity (Peate et al., 2002). There is potentially going to be a move towards more occupation related fitness testing and literature informs that testing firefighters under actual work conditions i.e. in full PPE and SCBA is likely to be more accurate. Furthermore, cardiopulmonary fitness level is inversely associated with risk of developing cardiovascular disease (Myers et al., 2015, DeFina et al., 2015). Studies have found that regular physical activity and a higher fitness level would offset the physiological strain encountered during firefighting (Ozemek et al., 2015) and also offset the risk of sustaining an injury (Poplin et al., 2013). With an increase in aerobic capacity of just one

MET, there is a decrease of 13 % in all-cause and 15 % in cardiovascular mortality (Kodama et al., 2009). Lower cardiopulmonary fitness amongst firefighters is also associated with abnormal exercise test results such as chronotropic insufficiency and ECG abnormalities which may indicate a higher future risk of cardiovascular events (Baur et al., 2012b).

1.2.2 OBESITY

Obesity is one of the most poorly addressed cardiovascular risk factors in the general population and is also a growing concern within the fire service. Studies have revealed that the prevalence of class 1 obesity (body mass index [BMI] >30) ranges from 32-40 % with 2 % prevalence of class 2 obesity (BMI >35) amongst a cohort of American firefighters (Kales et al., 1999a, Soteriades et al., 2005, Tsismenakis et al., 2009, Poston et al., 2011). The same studies revealed that 77-90 % of American firefighters would be categorised as being overweight (BMI 25-30) or obese with a prevalence exceeding the general US population (Geibe et al., 2008). Comparatively in the UK, 65 % of firefighters were classified as being overweight or obese compared with 45 % of the general population (Munir et al., 2012). Alarmingly, in a cohort of firefighters prospectively followed up over a 5-year period, the prevalence of obesity and morbid obesity (BMI >40) increased with time and on average firefighters gained 1.15 pounds per year, gaining weight at a higher rate in the latter years of active duty (Soteriades et al., 2005). This is not altogether unsurprising when we consider that the job is

characterised by prolonged periods of low-intensity work or in a sedentary state.

Obesity amongst military personnel was found to be associated with lower aerobic capacity and decreased muscular endurance (Jette et al., 1990). In firefighters, obesity has been shown to correlate with higher systolic and diastolic blood pressure levels, higher total cholesterol levels and a lower cardiopulmonary fitness level (Clark et al., 2002) and to higher levels of highly sensitive C-reactive protein (hs-CRP) and a lower Buckberg sub-endocardial viability ratio which is indicative of a reduction in supply to demand ratio of oxygen in the coronary arteries (Smith et al., 2012). Increased BMI was found to be a major driver of increased LV mass, a strong predictor of cardiovascular events, and has been shown to be reversible with weight loss (Korre et al., 2016). Furthermore, obese firefighters are more likely to experience the adverse consequences of heat stress.

1.2.3 AGE

In the United States the mean age of firefighters dying of a coronary heart disease event was 50.4±7.6 years (Geibe et al., 2008). Due to age-related changes in physiology and a lengthened exposure to potential risk factors, older persons in general are at higher risk of cardiovascular disease. Older

firefighters are similarly at higher risk of cardiovascular disease. Indeed, the risk of cardiovascular events has been demonstrated to increase sharply beyond 60 years of age (Kales et al., 2007, Holder et al., 2006). On-duty cardiovascular death amongst firefighters age 60 and above is 4 to 18 times more likely than in colleagues aged 40-49 years. This issue is likely to become more pertinent, especially in the UK, in years to come with an ageing fire service following a decision from the UK government to increase the firefighter national pension age from 55 to 60 years of age. Studies have already reported that the age-related decline in cardiopulmonary fitness is no different to the general population and factors such as higher BMI and self-reported lack of physical activity accelerate this decline (Baur et al., 2012a). Such decrements in cardiopulmonary fitness can, however, be attenuated by maintenance of regular physical activity and maintaining a healthy weight.

1.2.4 Traditional cardiovascular risk factors

Firefighters, despite being mostly healthier than the general population, are not immune to the traditional cardiovascular risk factors that effect us all. Cigarette smoking amongst firefighters is unsurprisingly less prevalent than in the general population with 12 % of American firefighters reported to smoke (Kales et al., 1999) compared with 15 % of American adults (CDC). However, with regards to other modifiable cardiovascular risk factors such as high blood pressure, high blood cholesterol and diabetes, a number of

studies are now reporting that the prevalence of these risk factors are similar to the general population (Wolkow et al., 2014). Furthermore, the prevalence of elevated blood total cholesterol and blood pressure exceeded the healthy people 2010 targets and were higher than in the general population (Byczek et al., 2004).

Hypertension in firefighters has a prevalence ranging from 11-23 % (Choi et al., 2016, Soteriades et al., 2003) with one study demonstrating that firefighters who had worked extra 24-h shifts and reported increased job demands were risk factors for hypertension amongst firefighters (Choi et al., 2016). Medical surveillance has been found to be effective in detecting hypertension amongst firefighters however when followed up over time only 42 % of firefighters were receiving treatment and 74 % of firefighters had inadequately controlled hypertension (Soteriades et al., 2003). Conversely, in a similar medical surveillance program assessing hypercholesterolaemia, cholesterol levels declined significantly over time and treatment rates improved. However, a considerable number of firefighters had persistently elevated cholesterol without adequate treatment (Soteriades et al., 2002).

Hypertension and current smoking were also both found to be strong predictors of cardiovascular death amongst firefighters (odds ratios 4.15 and 3.68 respectively) (Geibe et al., 2008). The level of hypertension is also important with an odds ratio of 3.2 for treated stage II hypertension (≥

160/100mmHg) and 4.6 for untreated stage II hypertension (Kales et al., 2002). Worryingly, the risk profiles of firefighters who had retired due to on-duty cardiovascular events were the same as those whose cardiovascular disease had manifested itself off-duty suggesting there is no easy way of determining whom might be most at risk of an on-duty event (Holder et al., 2006).

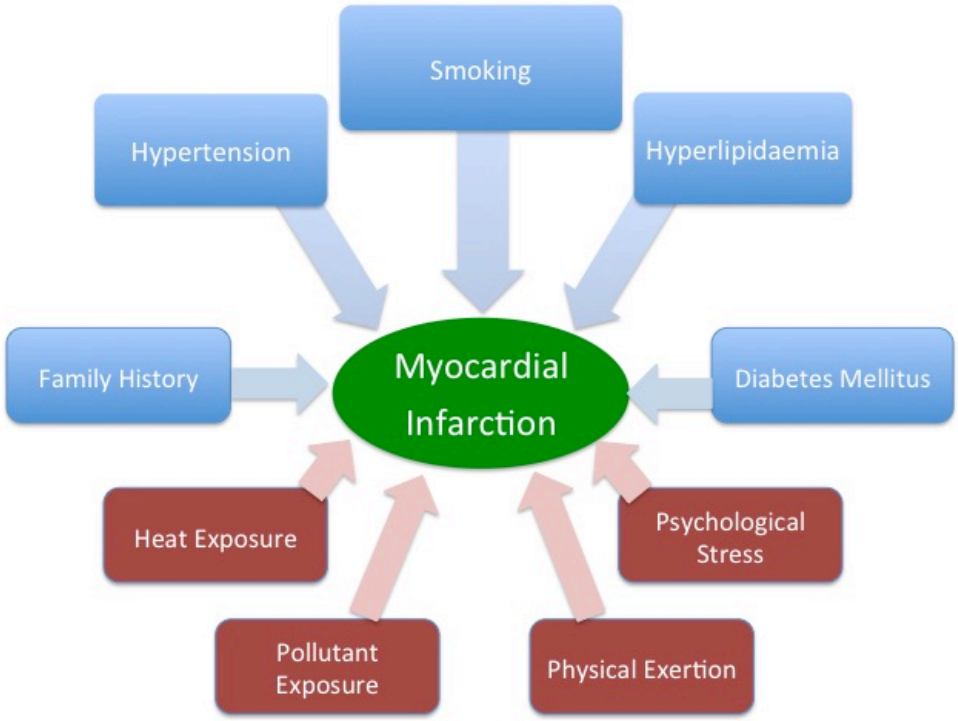
1.2.5 Pre-existing Coronary Artery Disease

The majority of firefighters dying on-duty from cardiovascular disease had underlying coronary artery disease (CAD), although only 18-26 % of firefighters had a pre-existing diagnosis of CAD or other arterial disease (Kales et al., 2003, Holder et al., 2006). In a cohort study of American firefighters, more than half had at least one high risk electrocardiogram (ECG) feature present reflecting possible CAD and one third had an abnormal exercise ECG requiring further evaluation to rule out subclinical CAD (Al-Zaiti and Carey, 2015). Other studies assessing firefighters with abnormal exercise ECG findings for evidence of subclinical coronary artery disease by computed tomography (CT) revealed that between 36 % and 53 % of active firefighters had coronary artery calcification (Superko et al., 2011, Pillutla et al., 2012, Santora et al., 2013). When compared to a matched group of the general population, firefighters had greater coronary calcium scores with a higher than average Agatston score recorded in 87%

firefighters compared with a national database and an Agatston score above the 75th percentile recorded in 57 % of firefighters (Santora et al., 2013). However, it is important to acknowledge that this particular study was limited by selection bias as CT scans were performed based on clinical indication and not systematically. Another study revealed firefighters had more left main stem and left anterior descending artery lesions (Pillutla et al., 2012). The presence of coronary calcification also correlated with other cardiovascular risk factors (Superko et al., 2011).

A pre-existing diagnosis of CAD is strongly correlated to cardiovascular death amongst firefighters (odds ratio 4.09) (Geibe et al., 2008). Moreover, autopsies of firefighters who have died on duty have typically shown varying degrees of coronary atherosclerosis usually accompanied by left ventricular hypertrophy (Geibe et al., 2008, Yang et al., 2013) with one study showing that cardiomegaly was associated with a five-fold increase in sudden cardiac death (Yang et al., 2013).

Figure 1-1 Occupational and Traditional Cardiovascular Risk factors for Myocardial Infarction in Firefighters



Traditional cardiovascular risk factors (blue) and occupation-related risk factors (red) with a combination of all or some culminating in a myocardial infarction.

1.3 Triggers for Myocardial Infarction in Firefighters

Although pre-existing traditional risk factors for cardiovascular disease are important to identify and manage within firefighters, the occupational risk factors inherent to the job are equally important to recognise and if completely unavoidable then efforts should be proportionately focused on reducing their impact. Furthermore, gaining understanding of the interaction of such occupational risk factors or triggers for cardiovascular events relevant to firefighters is key to reducing the incidence of such events and should be the priority of the fire service, the medical community, and society as a whole.

1.3.1 Extreme Physical Exertion

Firefighters are exposed to an episodic pattern of intense physical exertion compounded by the lifting of heavy equipment and materials. The breathing apparatus required to be worn during firefighting weighs 24 kg and can be worn for prolonged periods. Strenuous physical exertion is known to independently trigger sudden cardiovascular events, particularly in individuals unaccustomed to exercise (Mittleman et al., 1993), despite the accepted role of regular aerobic exercise in prevention and rehabilitation. As aforesaid, firefighters have long periods of low-intensity work or in a sedentary state interspersed with occasional bouts of moderate to high intensity effort.

Strenuous physical exertion leads to higher heart rate and blood pressure, increased myocardial oxygen demand and activation of the sympathetic nervous system. Each episode of physical exertion is associated with a transient increased risk of myocardial infarction and sudden cardiac death (Mittleman and Mostofsky, 2011). Two previous large-scale epidemiological studies have determined that risk is 5.9-6.1 times higher within the immediate period following exercise compared with periods of lower levels of activity or rest (Mittleman et al., 1993, Hallqvist et al., 2000). The relative risk is greater amongst those who reported undertaking less than 1 episode of heavy activity

In an example of a fire simulation exercise, subjects ascend stairs dragging a hose, to locate and extinguish a fire and rescue an 80 kg dummy (Angerer et al., 2008).. Search and rescue within a fire suppression operation is generally regarded as the most physically demanding activity a firefighter can undertake. On this occasion temperatures in the rooms reached 200 °C at 1.5 m above ground, 470 °C below the ceiling after 3 minutes and attained maximum values of 700 °C with a mean duration of 29±5 min per exercise. The heart rates of the majority of participating firefighters markedly exceeded those attained in recruitment exercise tests and 14 % of firefighters attained a heart rate higher than their age predicted maximum. Firefighting activities in similar simulated conditions increased heart rate to close to or above age-predicted maximal levels in other studies. (Manning and Griggs, 1983, Del

Sal et al., 2009, Smith et al., 2001). A handful of studies have monitored heart rates during actual emergencies and report sharp increases in heart rate, approximately 60 beats per minute (bpm), a combination of sudden increase in activity in making way to fire engine and the response to the fire station alarm (Sothmann et al., 1992, Barnard and Duncan, 1975, Bos et al., 2004). Heart rates continued to increase (both peak and average) during successive bouts of activity (Horn et al., 2013a), even with rest periods of between 20 and 35 min. This is concerning considering that actual real-life firefighting may entail multiple successive bouts of firefighting with less rest or time for recovery in between.

Despite the apparent strenuous exertion, on average, the training exercise was graded by the firefighters as *strenuous yet not very hard* on the Borg Scale (Angerer et al., 2008b). Ratings of perceived exertion are commonly used in simulated real fire exercises. However, there appears to be poor correlation between perceived exertion and heart rate with most subjects grading exercises as less strenuous than their heart rates would otherwise suggest (Smith et al., 1996, Smith et al., 2001). This raises an important safety issue and questions if firefighters are aware they are working at the limits of their physiological capabilities.

Firefighters with greater cardiopulmonary fitness levels have demonstrated better air ventilation with breathing apparatus (Gendron et al., 2015) so may

potentially be able to prolong cylinder use increasing safe period within difficult situations such as a search and rescue within a burning building. Another vital role of increased fitness in offsetting not just cardiovascular risk but improving safety.

1.3.2 Heat Stress: Increased core body temperature

Heat stress contributes strongly to cardiovascular strain and results from the external stress of heat radiating from the fire and exercise-induced metabolic heat stress. Moreover, firefighting protective equipment is multi-layered and insulated, further exacerbating the challenge of thermoregulation. Indeed, studies have shown that exercise in full PPE for a prolonged period of time even in an ambient environment can result in temperature rises. Baker et al demonstrated that working for 60 min in full PPE in an ambient temperature of 21±1.5 °C (relative humidity 55±5 %) resulted in a mean temperature rise of 1.3 °C to 38.4 °C (Baker et al., 2000). However, in this study, working at moderate intensity in the same temperature for a shorter duration (12 min) there was no change in core body temperature wearing full PPE as compared to wearing sports gear. The heat stress associated with firefighting causes vasodilation and combined with associated fluid losses due to profuse sweating, results in eventual reduced cardiac output and a hypercoagulable state (Smith et al., 2001, Angerer et al., 2008).

The safe upper limit for an increase in core temperature is set at 1.4 °C per hour during fire suppression activity (Ergonomic Standard ISO 9886). This limit was exceeded by 12 % of firefighters with the average core body temperature measuring 38 °C during exercise (Angerer et al., 2008). This measured mean increase in temperature was in-line with other studies (Sothmann et al., 1991, Smith et al., 1996, Smith et al., 2001). Smith et al found a slightly higher maximum core temperature of >39 °C in 18 % of subjects in a similar study (Smith et al., 2001).

Heat stress also limits the period of effective work, which has major implications for firefighters. Prior to increases in core temperature (when only skin temperature is elevated), reductions in self-selected exercise work rate in the heat are likely mediated by thermal perception (thermal comfort and sensation) and its influence on the rating of perceived exertion (Flouris and Schlader, 2015). When both core and skin temperatures are elevated, factors associated with cardiovascular strain then dictate the rate of perceived exertion response. Time to exhaustion has been found to be inversely related to the rate of heat storage. However, thermal perception is not strongly correlated with measured core body temperature (Savage et al., 2014) suggesting that perception alone is an unreliable source of information to guide decisions on heat stress. Interventions such as active cooling can counteract heat storage and has been shown to decrease physiologic strain and lead to longer periods of effective work (Carter et al., 1999b). It has also

been demonstrated that firefighters, who have predominantly been fighting wildland fires over a 4-month period, display features of heat acclimatisation (Lui et al., 2014).

The core temperature and rate of rise in temperature increases with longer duration firefighting activities. Furthermore, core temperature, rate of temperature rises and heart rate all continue to increase with subsequent exposures. Higher BMI and higher resting HR was associated with lower heart rates, core temperature and rate in temperature rise during exposures. This has been demonstrated in an earlier study where an increased waist circumference was also associated with a lower core body temperature rise during exposure when compared with those with a smaller waist circumference. In the same study, those who exercised a greater number of hours per week or who had a lower low-density lipoprotein level had a greater increase in core body temperature (Burgess et al., 2012). This suggests that those subjects with a lower BMI, smaller waist circumference or a more favourable cardiovascular condition exerted themselves more than less fit firefighters.

Peak in core temperature tends to lag far beyond the exiting the simulated fire environment (Smith et al., 2001, Hostler et al., 2009, Smith and Petruzzello, 1998) and has varied from 5-11 min (Horn et al., 2013) and up to 15 min thereafter (Burgess et al., 2012). This supports a minimum duration

of rehabilitation of 10-15 min following simulated fire suppression. Furthermore, the recovery of heart rate and core temperature is strongly correlated with time between work cycles and is unrelated to individual descriptive characteristics or the previous amount of work completed (Horn et al., 2013). The average rate in core temperature reduction during rest was approximately half of the average rate of core temperature increase during firefighting activities (Horn et al., 2013) with recovery of heart rate and core temperature returning to baseline 50-80 min after firefighting activity ceased (Horn et al., 2011). Older firefighters display similar rates of heat loss to age-matched non-firefighters and both groups have a slower reduction in core body temperature than younger firefighter counterparts (Kenny et al., 2015).

1.3.3 Psychological Stress

The psychological pressures of fire-fighting are inherent. The main contributors are time pressures, being awoken by alarms, being called into unforeseeable circumstance with unpredictable risks and the burden of being responsible for the lives of others. Abrupt onset psychological stress is known to lead to increased heart rate, blood pressure and therefore myocardial oxygen demands and leads to activation of the sympathetic nervous system. Acute psychological stress has also been shown to stimulate of inflammatory cytokines and platelet aggregation (Strike et al., 2006, Strike and Steptoe, 2005), with sleep disturbances in particular

associated with changes in interleukin-6 production and higher levels of sympathetic tone and cortisol output overnight (Tofler et al., 1987).

Research has identified increased heart rates in firefighters responding to alarms, especially when awoken from sleep, consistent with sympathetic stimulation. Noise exposure and psychological stress when attending emergencies will also increase heart rate and blood pressure further. A previous meta-analysis of noise exposure and cardiovascular outcomes revealed an association between noise exposure and hypertension with a notable positive association between road traffic noise exposure and ischaemic heart disease (van Kempen et al., 2002).

Anxiety levels have been assessed, using validated psychological measurements, following real-fire training and are elevated within a hot environment compared with the same training exercise in ambient temperatures (Smith et al., 1997). Anxiety levels remained raised at 10 min following the exercise. Elevated anxiety levels may impact on cognitive functioning and may result in inappropriate decisions. Furthermore, Smith *et al* demonstrated a reduction in accuracy of firefighters performing three 7 min simulated fire-suppression exercises in quick succession, yet there was no decline in reaction time (Smith et al., 2001). Subjects made progressively more mistakes in the latter exercises.

Moreover, the combination of physical and psychological challenges experienced by firefighters, referred to as a dual-stress condition, resulted in exacerbated responses of stress hormones such as adrenaline, noradrenaline and the hormones of the hypothalamic-pituitary-adrenal axis in a simulated fire training scenario (Webb et al., 2011) thus providing another potential mechanism that could contribute to increased cardiovascular events in firefighters.

1.3.4 Air Pollution

Globally, the World Health Organization estimates that urban air pollution contributes to 5 % of all cardiopulmonary deaths which translates into around 100,000 premature deaths in Europe and 1.3 million deaths worldwide each year (WHO Air Quality Guidelines, 2005). Firefighters may be exposed to appreciable concentrations of a range of toxic materials during routine occupational activities. An early study by Brandt-Rauf and colleagues demonstrated that firefighters are frequently exposed to carbon monoxide, benzene, sulphur dioxide, hydrogen cyanide, aldehydes, hydrogen chloride, dichlorofluoromethane and particulate matter, all at levels above the personal exposure limit or time weighted average for 8 h in at least half of the fires assessed (Brandt-Rauf et al., 1988a).

Air pollution can be defined as a heterogeneous and dynamically changing mixture of gases and particulate matter (PM) that makes it difficult to study any individual component in isolation. Over 98% of combustion-derived pollutant mass is from gases or vapour phase compounds such as carbon monoxide, volatile organic carbons, the nitrogen oxides and sulphur dioxide. However, particulate matter is the most strongly associated with adverse health effects. Particulate matter is categorized, monitored and regulated on the basis of its aerodynamic diameter. Particles with a diameter of less than 10 μm can be inhaled deep into the lungs and are quantified by mass as $PM_{10.}$ Smaller particles of less than 2.5 μm diameter are referred to as $PM_{2.5}$ or fine PM, whilst particles of less than 0.1 μm diameter are ultrafine, nanoparticles or $PM_{0.1}$. The current WHO Air Quality Guideline targets for annual mean exposure of $PM_{2.5}$ and PM_{10} are 10 and 20 $\mu g/m^3$ respectively. Typical background concentrations of PM_{10} in North America and Western Europe are between 20-50 $\mu g/m^3$ whilst in industrialised areas and in the developing world are between 100 and 250 $\mu g/m^3$. The toxicity of PM relates to the number of particles encountered, together with size, surface area and chemical composition. Occupational exposures to air pollution differ from those of the general population in both composition and concentration of air pollution, exposure frequency and duration of exposure (i.e. shift work). Occupational exposures are generally higher than ambient levels. Hence this group may be most at risk.

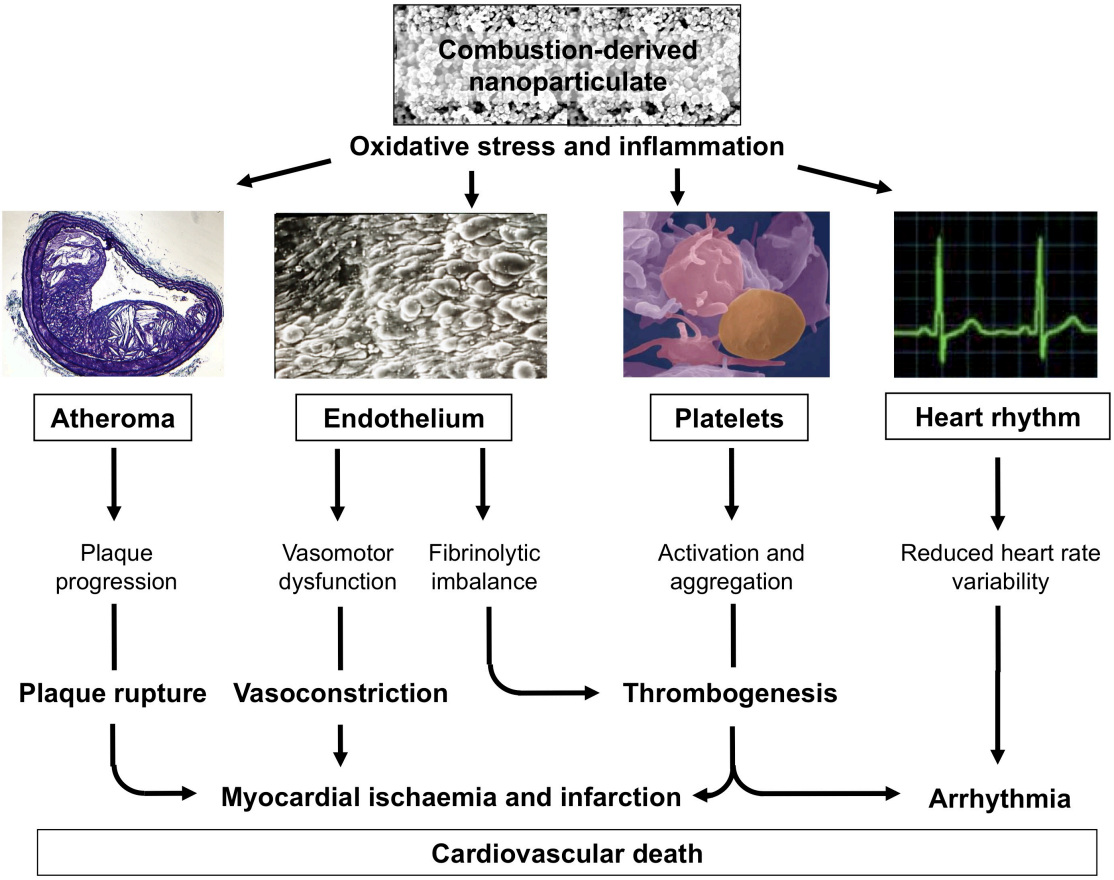
Air pollution is increasingly being recognised as an important and modifiable risk factor for cardiovascular disease. Owing to the pervasive nature of air pollution, millions of susceptible individuals are unknowingly at greater cardiovascular risk: hence its rightful position as a major public health concern. Indeed, Nawrot and colleagues evaluated potential triggers of myocardial infarction revealing that both exposure to traffic and to a 30 $\mu g/m^3$ change in PM_{10} had the greatest population effect in triggering cardiovascular events (Nawrot et al., 2011).

In addition to the effects of air pollution as an independent cardiovascular risk factor, it also appears to adversely affect other traditional risk factors. Brook and colleagues (Brook et al., 2008) demonstrated a relationship between the long-term traffic exposure and the odds of having a diagnosis of diabetes mellitus, whilst Zanobetti and colleagues (Zanobetti et al., 2004) reported acute increases in systolic blood pressure in relation to higher daily ambient PM concentrations.

There are two widely accepted hypotheses as to how particulate matter air pollution may increase cardiovascular risk. The original hypothesis proposed that inhaled particles provoke an inflammatory response in the lungs, with consequent release of pro-inflammatory cytokines into the circulation (Seaton et al., 1995). Alternatively, inhaled, insoluble ultrafine PM or nanoparticles could rapidly translocate to the circulation via the alveolar-blood barrier and

interact directly with the vascular endothelium or atherosclerotic plaques causing local oxidative stress and pro-inflammatory effects similar to those seen in the lungs (***Figure 1.2***). Experimental studies in man and animal models demonstrate direct translocation of a small fraction of ultrafine particles from the alveoli into the circulatory system (Oberdorster et al., 2005). Miller, Raftis and colleagues recently demonstrated the translocation of inhaled nanoparticles of gold, a model particle chosen with a distinct signal that could be tracked into blood and urine in healthy male volunteers and has been detected in carotid plaque in patients with carotid stenosis undergoing carotid endarterectomy, following inhalation (Miller et al., 2017).

Figure 1-2 Mechanisms of Cardiovascular Effects of Air Pollution



Postulated pathways by which inhaled nanoparticles exert cardiovascular effects and culminate in cardiovascular events (Hunter et al., 2012, Mills et al., 2009).

1.3.4.1 WOODSMOKE

Wood smoke has for many years been viewed as a relatively benign substance owing to its natural origins. However, it is now well established that wood-burning fires emit thousands of health damaging pollutants including free radicals, CO, and nitrogen oxides as well as ciliatoxic respiratory irritants such as phenols, cresols, acrolein, and acetaldehyde; carcinogenic organic compounds such as benzene, formaldehyde, and 1,3 butadiene; carcinogenic cyclic compounds such as polyaromatic hydrocarbons (PAHs) and particulate matter (PM) (Dubick et al., 2002, Leonard et al., 2000, Naeher et al., 2007, Larson and Koenig, 1994). Combustion of wood is typically inefficient and partially oxidised organic chemicals are produced. Organic tracers for smoke from incomplete combustion of wood include levoglucosan from cellulose and methoxyphenols from lignin; both of which are potential biomarkers for exposure.

Fresh wood smoke contains large quantities of ultrafine particulate matter (PM) by which the most significant threat of air pollution is posed. Wood smoke particles are generally smaller than 1 μm with a size distribution of 0.15 to 0.4 μm (Hays et al., 2002). Biomass combustion is common in residential settings globally as a means of cooking and heating. Firefighters however, have arguably the most significant exposures during wildland

firefighting. Wildland fires are becoming ever more hazardous due to climate change along with an increase in frequency and intensity (Withen, 2015). The mean $PM_{2.5}$ exposure during this setting was 280 μg/m^3 for burn day samples, and 16 μg/m^3 on non-burn days. Overall occupational exposures to particulate matter are low, but exposure could exceed the recommended threshold limit value of 3 mg/m^3 for respirable particulate matter in a few extreme situations. (Adetona et al., 2011). Additionally, Leonard et al (Leonard et al., 2000, Leonard et al., 2007) demonstrated in wood smoke that coarse size-range particles contained more carbon radicals per unit mass than the ultrafine particles, however, the ultrafine particles generated more hydroxyl radicals in an acellular Fenton-like reaction. The ultrafine particles also caused significant increases in hydrogen peroxide production by monocytes and lipid peroxidation.

Woodsmoke, particles are characterised by a larger mean particle diameter but smaller surface area to mass than vehicle exhaust particles (Kocbach et al., 2006). Woodsmoke also has a higher content of organic carbon and PAHs. Particle toxicity is known to increase with surface area (Brown et al., 2001) while a high content of organic carbon and PAHs has been associated with increased inflammatory and oxidative potential (Li et al., 2003).

Woodsmoke particles can also be transported over hundreds of kilometres as they are not easily removed by gravitational settling with consequent

effects at a general population level following forest fires (Naeher et al., 2007). For example, during fires in California, hourly $PM_{2.5}$ peaked at 160 $\mu g/m^3$ and PM_{10} peaked at 200 $\mu g/m^3$ at the nearest monitoring site, concentrations greatly in excess of the California 24-h average ambient air quality standards ($PM_{2.5}$ 35$\mu g/m^3$ and PM_{10} 50 $\mu g/m^3$) (Wegesser et al., 2009)

1.3.4.2 STRUCTURAL FIRES

Due to the heterogeneity of the materials involved in structural fires, the smoke particles in this instance are difficult to characterize with any consistency. Concentrations of combustion products vary tremendously from fire to fire depending upon the size, the chemistry of materials involved, and the ventilation conditions of the fire. However, ultrafine particles again represent the most prevalent type of PM, generated during combustion of common residential materials and products (Baxter et al., 2010). The density of ultrafine particles in urban air ranges from $5x10^3$ to greater than $3x10^5$ particles/m^3 (McMurry and Woo, 2002) with values in the upper part of this range are therefore comparable to those found during fire suppression in a large study assessing experimental structural fire situations (Fabian et al., 2010). Higher particle number densities were observed for the room fires than the external decks and automobile fires, presumably due to containment within an enclosed space. However, despite differences in furnishings and

other items (and their chemical compositions) involved in the fires, the smoke particle size distributions were found to be similar for all seven scenarios with particles <1 μm in diameter comprising >99 % of those measured (Fabian et al., 2010).

Specific potential exposures to other toxins can include metals such as lead, antimony, cadmium, and uranium; chemical substances, including acrolein and benzene; polyaromatic hydrocarbons (PAHs), perchlorethylene, toluene, trichloroethylene, trichlorophenol, xylene, formaldehydes, minerals such as asbestos, crystalline, and noncrystalline silica, silicates, and various gases that may have acute, toxic effects (Fabian et al., 2010). A recent study also found high levels of di-(2-ethylhexyl) phthalate (DHEP), a plasticiser and probable carcinogen on firefighter clothing (Alexander and Baxter, 2014)

It was suggested that modern-day building materials (polymers) might give rise to new supertoxicants, either during the phase of rapid combustion (knockdown) or during the smouldering conditions following extinction of the fire (overhaul). The analysis of samples obtained at experimental fires and at municipal structural fires found the same substances (propene, isoprene, benzene, toluene, ethyl- benzene, styrene, propene, and 1,3-butadiene) at high concentrations relative to other combustion products. Together with naphthalene, these degradation products of polymeric material were also the principal combustion products of wood, the predominant construction

material in the past (Austin et al., 2001). Benzene levels were highest at fires involving wood structures (Brandt-Rauf et al., 1988b) . Two studies found many of the same contaminants during overhaul as had previously been found during knockdown but at much lower levels, with the exception of aerosolized building materials (Jankovic et al., 1991, Bolstad-Johnson et al., 2000). Lower combustion temperatures characteristic of the latter stages of a fire do not appear to result in higher levels of toxic combustion products. Furthermore, the spectra of combustion products were similar at mixed-occupancy, municipal structural fires, an electronics industry fire and, a 9-day smouldering fire (Austin et al., 2001).

1.4 EVOLUTION OF CARDIOVASCULAR EVENTS IN FIREFIGHTERS

1.4.1 PATHOGENESIS OF ATHEROTHROMBOSIS

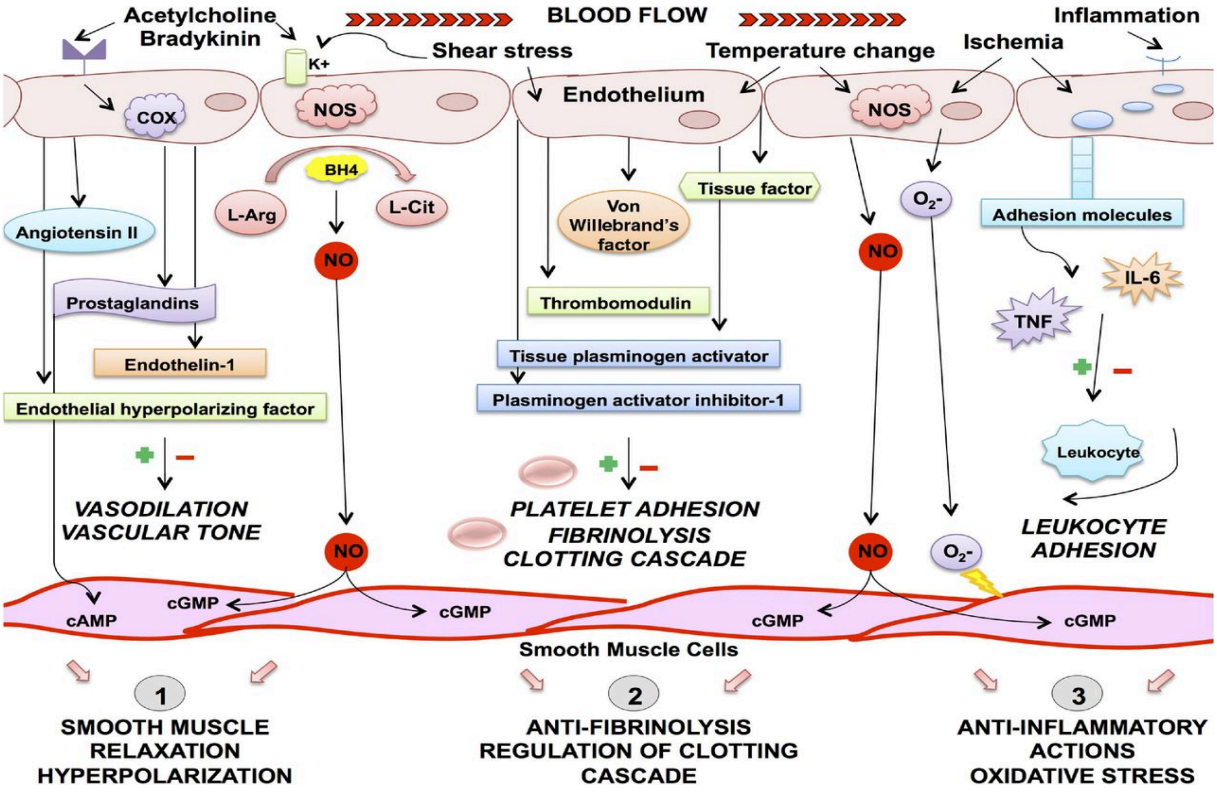
Atherothrombosis is characterised by disruption of an atherosclerotic plaque with thrombus formation and is the major cause of acute coronary syndromes and cardiovascular death (Fuster et al., 2005). Dysfunction of the vascular endothelium is one of the earliest pathological features of atherosclerosis (Ross, 1999), with loss of endothelial integrity resulting in expression of leucocyte adhesion proteins, reduced anti-coagulant activity and platelet activation. The endothelium contains three thromboregulators: nitric oxide, prostacyclin and the ectonucleotidase CD39, which together in health, provide a robust defence against thrombus formation (***Figure 1.3***). The vascular endothelium delicately balances these regulatory pathways controlling blood flow, coagulation, fibrinolysis and inflammation. Vascular endothelial dysfunction, which is generally characterised by the reduced bioavailability of endothelium-derived nitric oxide, has been shown to be an independent risk factor for cardiovascular morbidity and mortality (Schächinger et al., 2000, Al Suwaidi et al., 2000). It is widely recognized that a variety of risk factors including cigarette smoking can influence vascular tone through endothelium-dependent actions (Celermajer et al., 1993, Newby et al., 1999), and there is now extensive evidence of abnormal endothelium-dependent vasomotion in patients with atherosclerosis (Newby et al., 2001, Ludmer et al., 1986).

Intravascular thrombus formation is the major pathogenic process in acute cardiovascular events, especially within the coronary system. When the endothelium is breached, collagen and tissue factor become exposed to flowing blood, thereby triggering thrombus formation. The process is complex consisting of two complementary pathways: the contact activation pathway where platelet activation and aggregation is triggered by exposed collagen and von Willebrand factor (vWF), and the tissue factor pathway of the coagulation cascade where tissue factor generates thrombin, in turn converting fibrinogen to fibrin and further activating platelets. One or other pathway may predominate, but the consequences of platelet activation triggered by these pathways are the same. Thrombus formation is a dynamic process where some platelets adhere to and others separate from the developing thrombus, and where shear, flow, turbulence and number of platelets in the circulation greatly influence the architecture of the clot (Furie and Furie, 2008). Furthermore, platelet aggregation has been shown to be significantly increased between 6 to 9am (Tofler et al., 1987), when the incidence of myocardial infarction peaks in the general population.

The endogenous fibrinolytic system protects against intravascular thrombosis and is particularly important in the coronary circulation (Rosenberg and Aird, 1999). Indeed, the fibrinolytic capacity of the endothelium predicts future adverse cardiovascular events in patients with coronary disease (Robinson et al., 2007). Small areas of endothelial denudation and thrombus deposition

are a common finding on the surface of atheromatous plaques and are usually sub-clinical because processes, such as endogenous fibrinolysis, prevent thrombus propagation and vessel occlusion (Davies, 2000). However, in the presence of an adverse pro- inflammatory state or an imbalance in the fibrinolytic system, such microthrombi may propagate, ultimately leading to arterial occlusion and the clinical manifestations of this, myocardial infarction or sudden death (Rosenberg and Aird, 1999). Thus, the initiation, modification and resolution of thrombus associated with unstable and inflamed atheromatous plaques is critically dependent on the efficacy of endogenous fibrinolysis, itself dependant on cellular activation and function of the surrounding endothelium and vascular wall. The fibrinolytic factor tissue plasminogen activator (t-PA), through the generation of active plasmin from plasminogen, regulates the degradation of intravascular fibrin and is released from the endothelium through the translocation of a dynamic intracellular storage pool (van den Eijnden-Schrauwen et al., 1995). The efficacy of plasminogen activation and fibrin degradation is further determined by the relative balance between the acute local release of t-PA and its subsequent inhibition through formation of complexes with plasminogen activator inhibitor type I (PAI-I). This dynamic aspect of endothelial function and fibrinolytic balance is directly relevant to the pathogenesis of atherothrombosis.

Figure 1-3 Normal Endothelial Function



The endothelium is responsible for physiological functions such as: 1) regulation of vascular tone through balanced production of vasodilators and vasoconstrictors; 2) control of blood fluidity and coagulation through production of factors that regulate platelet activity, the clotting cascade, and the fibrinolytic system; and 3) regulation of inflammatory processes through expression of cytokines and adhesion molecules. cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; COX = cyclooxygenase; BH4 = tetrahydrobiopterin; IL = interleukin; TNF = tumour necrosis factor; L-arg = L-arginine; L-cit = L-citrulline; NO = nitric oxide; NOS = nitric oxide synthase; O_2- = superoxide. (Marti et al., 2012)

1.4.2 ACUTE CARDIOVASCULAR STRAIN OF FIREFIGHTING

Firefighting is characterized by repeated episodes of increased cardiac workload, compounded by dehydration and augmented sympathetic-adrenal stimulation (Horn et al., 2013). The occupational risk factors resulting in such physiological disturbances can be encountered individually for example, isolated physical exertion during a training drill. However, all other occupational risk factors are only ever encountered in combination during other firefighter duties. All identified occupational risk factors are encountered within a real-life fire suppression scenario and can adversely affect all body systems but in particular can lead to extreme cardio-circulatory and thermal strain (***Figure 1.4***).

The physiological strain of firefighting duties usually commences with alarm response which is known to cause sympathetic arousal along with increases in heart rate and blood pressure, increasing shear stress in blood vessels and increasing myocardial oxygen demand. Heart rate and blood pressure continue to increase until arriving at the scene then depending on the nature of the emergency, the physiological and particularly the cardiovascular strain can continue to escalate. In a fire suppression operation, for example, significant aerobic effort (stair and ladder climbing), anaerobic power (forcible entry, search and rescue operations) and static exertion (handling heavy materials or persons, cutting) are potentially required all whilst wearing heavy

and encapsulating PPE in extreme ambient temperatures. Collectively making fire suppression the most cardiometabolically demanding of all firefighter duties. Previous studies have demonstrated the deleterious physiological effects.

Simulated fire suppression offers a unique opportunity to assess the effects of extreme physical exertion and heat stress in combination in a controlled environment. Despite the controlled and often familiar situation of fire suppression simulation, studies have persistently demonstrated that firefighter heart rates approach and exceed age-predicted maximal heart rates and physiological limits, often for extended periods of time (Angerer et al., 2008, Al-Zaiti et al., 2015, Smith et al., 2001). This is in line with studies that have been able to monitor firefighter heart rates during real-life alarm response (Barnard and Duncan, 1975, Sothmann et al., 1992). Sustained heart rates between 180 beats per minute minus age in years and 220 beats per minute minus age for cardiovascular conditioned persons are also associated with excessive heat strain (Burgess et al., 2012).
Core body temperature has also been demonstrated to increase by around 1 °C. During a controlled study it was demonstrated that heat stress with an increase in core body temperature by 1.2° C caused a greater chronotropic effect than moderate dehydration (~3 % body mass loss) (Fehling et al., 2015).

Fire suppression is associated with an increase in blood pressure during the activity that quickly drops below resting values following the cessation of work (Horn et al., 2011). Healthy firefighters undertaking a week of repetitive training exercises in real-fire conditions demonstrated significant weight losses of approximately 1-1.7 % per day, signifying dehydration (Smith et al, 2004). Secondary to the fluid losses of profuse sweating, high metabolic demand and respiration, plasma volume is reduced by up to 15 % after only an 18 min fire simulation study. Somewhat unsurprisingly, stroke volume has also been shown to decrease following strenuous firefighting activity (Smith et al., 2001). In another study, a 30 % reduction in cardiac output was demonstrated in firefighters following three 7 min simulated fire-suppression exercises in quick succession (Angerer et al., 2008). Stroke volume decreased with each repeated exercise following an initial increase during the first task. There was, however, increased aortic peak acceleration and velocity suggesting that cardiac contractility remains enhanced during this strenuous exercise. A controlled exposure study assessing heat-stress and physical exertion in conditions of euvolaemia and hypovolaemia for short periods failed to demonstrate evidence of myocardial dysfunction on echocardiogram (Fehling et al., 2015). By contrast, a significant decline was observed in left ventricular dimension, shortening fraction and transmitral ventricular filling velocity with a reduced longitudinal relaxation rate in the lateral wall following repeated bouts of simulated fire suppression over a 3-h period (Fernhall et al., 2012). It was unclear whether these findings

represented dehydration or myocardial dysfunction. Furthermore, during another fire simulation pathological ST depression was demonstrated in a quarter of subjects together with poor heart rate variability (HRV) in over half during or after fire suppression in firefighters at low risk of cardiovascular disease (Al-Zaiti et al., 2015). Simulated fire suppression has been accompanied by an appreciable decrease in blood pressure with a significant number of subjects demonstrating postural hypotension, although all were asymptomatic (Angerer et al., 2008).

Non-invasive measurements of forearm blood flow and arterial stiffness were measured following 3 hours of simulated fire-suppression exercise (Fahs et al., 2011) in healthy young firefighters. There was an acute increase in aortic stiffness and augmentation index following the exercise. Such changes in arterial stiffness mirror those observed following heavy resistance exercise or supra-maximal anaerobic exercise. A simultaneous increase in microvascular function and carotid artery dilatation was demonstrated. Arterial stiffness is usually associated with impaired microvascular function and is independently associated with increased risk of cardiovascular event. The implications of these apparently opposing vascular effects remain unclear.

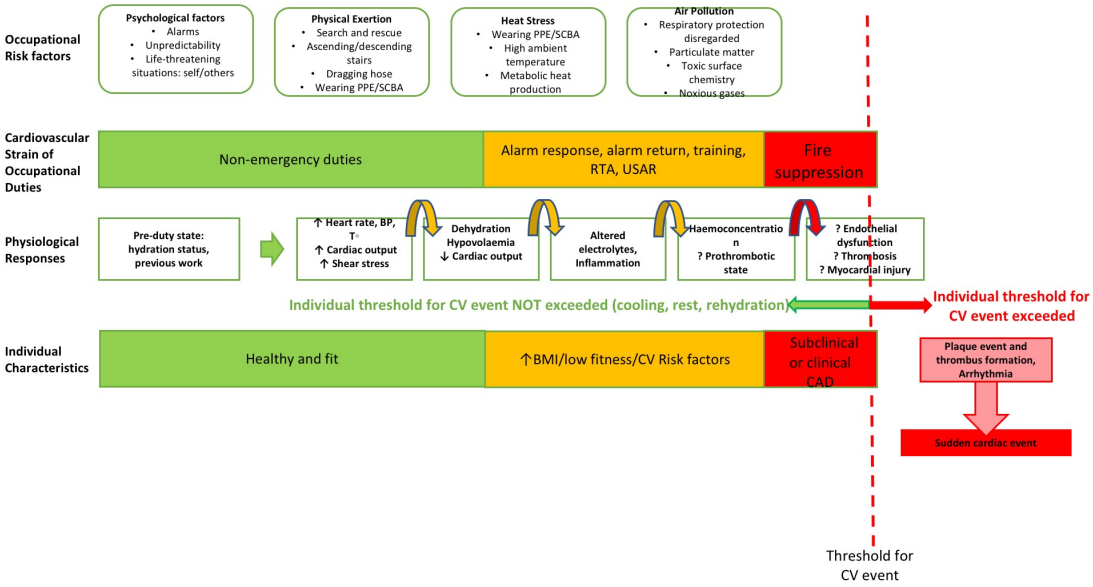
The decrease in plasma volume also results in haemoconcentration which has been shown to cause derangements in electrolyte homeostasis and increased blood viscosity (Smith et al., 2001). Simulated fire suppression

also increases platelet number and aggregation, and additionally activate clotting factors in firefighters, likely to be as a result of haemoconcentration (Smith et al., 2011). In a similar study, however, there was no increase found in platelet P-selectin or platelet-monocyte aggregates following exposure as determined by flow cytometry. However, in another study there was an increase in whole blood coagulability as measured by activated partial thromboplastin time (aPTT) and factor VIII even 2 h after exposure (Smith et al., 2013). An increase in thrombotic potential is normally accompanied by a parallel increase in endogenous fibrinolysis restoring a balanced haemostatic state. However, in the same study it was demonstrated that there were increases in both fibrinolytic and thrombotic activity as measured by t-PA and PAI-1 but that PAI-1 continued to remain elevated at 2 h post exposure suggesting an overall shift to a prothrombotic state.

Sustained strenuous exercise in isolation (even at normal ambient temperatures) causes a systemic inflammatory response as indicated by a leucocytosis, a polymorphonuclear CD11b increase and an increase in platelet-leucocyte conjugates (Peake et al., 2005). This effect is amplified when exercising in hyperthermic conditions with many studies demonstrating an increase in inflammatory markers following simulated fire suppression, most notably leucocytes (Burgess et al., 2012) and acute changes in blood chemistry. During an intensive week of daily simulated fire-suppression

exercises circulating leucocyte numbers were increased by approximately 100% within each day whilst blood chemistry changed with increases in urea and creatinine (Smith et al., 1997). Blood lactate also increases during fire-suppression. Hyperthermia also induces hormonal changes as demonstrated by increased levels of cortisol in the plasma, which is attributed to the activation of the sympatho-adrenal-cortical axis (Rhind et al., 1999) and can also account for the marked increase seen in circulating leucocytes.

Figure 1-4 Mechanisms of Myocardial Infarction in Firefighters



Postulated pathway by which pre-existing personal and occupational risk factors interact and result in increasing physiological strain with the potential for resulting cardiovascular events in a susceptible few. RTA: road traffic accident. USAR: urban search and rescue.

1.4.3 THE IMPACT OF AIR POLLUTION RELATING TO FIREFIGHTERS

Air pollution remains an understudied and ill-defined occupational risk factor in firefighters, probably as the exposures are so diverse, but also because exposures to real-life pollutants are hard to replicate or study in a controlled situation. In contrast to urban air pollution the mechanisms underlying adverse health effects of wood smoke inhalation have not been as intensively studied and the effects of fire smoke from structural fires even less so. However, the predominant component of both these types of fire is ultrafine PM.

Firefighters, during active fire suppression, are usually protected from smoke exposure by self-contained breathing apparatus (SCBA). However, this is often removed during overhaul (period following fire extinguishing). In other situations respiratory protection is often inadequate or disregarded resulting in potentially hazardous but tolerable situations, such as wildland fires where the long duration and remote location of firefighting often renders SCBA wearing impractical (Bolstad-Johnson et al., 2000). Moreover, the physically demanding task of firefighting can result in substantial particle deposition within the respiratory tract, reflecting a combination of increased respiratory rate and consequently minute ventilation together with obligate mouth breathing and loss of nasal filtration. Additionally, smoke deposits and condensed residues accumulate on firefighting garments and skin from fire

exposure and remain on firefighter PPE and skin until cleaned. These contaminants may lead to further exposure to firefighters and other individuals that come in contact with the firefighter PPE by inhalation and potentially also by skin absorption.

Ultrafine particles seem to be the most physiologically and toxicologically active component of fire smoke. Owing to their size they can evade the mucociliary defence systems and are deposited deeply into the alveolar spaces where they can remain indefinitely causing morphologic and biochemical changes (Araujo and Nel, 2009). The large surface area to mass ratio of these particles enables the transport of large amounts of adsorbed toxic agents to internal targets (Wittmaack, 2007). Such toxins as carbon monoxide independently cause cardiac ischaemia (Allred et al., 1989). Additionally, quinones, a special group of carbonyl containing PAH compounds are particularly reactive organic components of PM with potential to produce reactive oxygen species and to induce oxidative stress via their redox capacity (Kocbach Bolling et al., 2009). The adsorbed gases on the surfaces of PM, such as volatile organic compounds, are potentially mutagenic with some having as much as 100 times the activity of some well-known carcinogens (Pierson et al., 1989).

Ultrafine PM is pro-oxidant and the proposed mechanisms of toxicity centre around lung inflammation via activation of alveolar macrophages and lung

epithelial cells. Systemic inflammatory responses are apparent with an increased presence of several cytokines (interleukin-6, interleukin-1b) in the bloodstream as well as increased production and release of granulocytes and monocytes from the bone marrow (Fujii et al., 2002, Goto et al., 2004). Furthermore, there is interference of autonomic control which some postulate is in response to direct reflexes from receptors in the lungs to the particles and/or to local or systemic inflammatory stimuli (Schulz et al., 2005). The cardiovascular effects, seen with exposure to other forms of nanoparticulate, such as diesel exhaust, have been demonstrated very early after exposure, and long before a significant systemic inflammatory response can develop. Therefore, translocation of these particles from the airways into the circulation and lymphatics with direct effects on the vasculature by particle constituents has also been suggested as a component for ultrafine particle toxicity (Furuyama et al., 2009, Wiebert et al., 2006).

Several *in vitro* studies of cultured cells have shown woodsmoke PM increases expression and production of pro-inflammatory cytokines, oxidatively damaged DNA and oxidative stress (Danielsen et al., 2008, Kocbach et al., 2008, Danielsen et al., 2011). Furthermore particles from incomplete combustion of woodsmoke induced more severe effects on both cytotoxicity and DNA damage *in vitro* than particles from more complete combustion conditions (Kocbach Bolling et al., 2009). A number of animal studies have shown that short term inhalation can compromise pulmonary

immune defence mechanisms, mainly through impairment of alveolar macrophage activity; cause oxidative stress and alter pulmonary histology causing bronchiolitis, parenchymatous blood vessel congestion and mild emphysema (Dubick et al., 2002, Matthew et al., 2001, Fick et al., 1984, Wegesser et al., 2009).

A number of exposure studies in healthy human subjects have shown similar increases in markers of systemic and lung inflammation. In one such study, following controlled exposure to wood smoke ($PM_{2.5}$ 240–280 μg/m^3 for 4 h) minor increases were seen in markers of systemic inflammation such as acute phase protein serum Amyloid A and to some extent serum C-reactive protein, in Factor VIII involved in blood coagulation and in urinary excretion of the isoprostane 8-isoPGF2 which is a marker of lipid peroxidation (Barregard et al., 2006). The oxidative DNA damage and related repair capacity in peripheral blood mononuclear cells was also investigated and although wood smoke exposure was followed by significant up-regulation of the repair gene hOGG1, no direct genotoxic effects were observed (Danielsen et al., 2008). In addition, there was increase in exhaled nitric oxide and Clara cell protein both serum markers of inflammatory effects on distal airways (Barregard et al., 2008). Similar increases in lung proteins were seen following real-life exposure to structural fires indicating increased lung permeability following smoke exposure (Burgess et al., 2002).

Firefighters assessed for evidence of pulmonary and systemic inflammation following wildland fire suppression showed increases in sputum neutrophils, circulating white blood cells and band cells (Swiston et al., 2008). Serum interleukin (IL)-6, IL-8 and monocyte chemotactic protein-1 levels also increased following fire-fighting which was not reported following strenuous exertion in isolation. Following smoke exposure from structural fires during overhaul IL-10 levels decreased acutely. IL-10 is involved in downregulating other inflammatory mediators (IL-8 and TNF-α) and macrophage expression of matrix metalloproteinases and in apoptosis of neutrophils (Burgess et al., 2002)

Urinary biomarkers of oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA), were measured in wildland firefighters revealing no change from pre- to post-shift levels. However, a change in 8-OHdG, corrected for renal function, from pre- to post-shift was associated with the length of firefighting career and subject's age suggesting that oxidative stress response may be dependent on age or the length of firefighting career (Adetona et al., 2012).

By contrast, Sehlstedt et al revealed virtually no effects on inflammation or oxidative stress in the airways after relatively large concentrations of $PM_{2.5}$ (224 ± 22 μg/m^3 for 3 h) (Sehlstedt et al., 2010). Although there was an increase in documented respiratory symptoms neither exhaled nitric oxide,

nor airway inflammatory parameters in bronchoalveolar lavage and bronchial mucosal biopsies were affected. Moreover, in atopic individuals, there was no effect on markers of oxidative stress, DNA damage, cell adhesion, or cytokines in response to clean air, low and high dose woodsmoke (14 μg/m^3, 220 μg/m^3, or 354 μg/m^3 respectively) (Forchhammer et al., 2012). Microvascular function assessed 6 h in the same study remain unchanged.

Exposure to fire smoke, may also have a cumulative effect in the genesis of atherosclerosis via systemic inflammation and oxidative stress. Long-term intermittent exposure to ultrafine particles with diameter <0.18 μm is implicated in the development of atherosclerosis in mice at a number density of 5,600 particles/m^3 (Araujo et al., 2008), which is comparable with levels found in structural fires (Baxter et al., 2010). However, no studies have assessed the long-term effects of fire smoke exposure.

Physical and chemical properties are likely to influence the biological potency of the particles and differences in this may account for the differing effects of fire smoke and other sources such as traffic pollution. This may also account for the differing effects of woodsmoke between studies which is likely to be due to different combustion conditions and appliances used.

1.4.4 THE FINAL COMMON PATHWAY LEADING TO CARDIOVASCULAR EVENTS

The combination of haemodynamic effects and pro-inflammatory state that fire suppression, and to a lesser extent, other firefighting duties can affect, sets the scene for endothelial dysfunction or increased plaque vulnerability which is further compounded by a prothrombotic state that increases the risk of thrombotic coronary occlusion and cardiovascular death. Not every firefighter has a cardiovascular event but they are arguably all at risk with marked physiological changes demonstrated in healthy firefighters. It is likely, therefore, that a threshold for a cardiovascular event is surpassed in specific firefighters who already have mitigating cardiovascular risk factors when they undertake more demanding firefighter duties, with fire suppression proving to be the ultimate challenge (**Figure 1.4**).

1.5 AIMS AND HYPOTHESIS

There are two principal aims addressed in this thesis. Firstly, I wish to gain a better understanding of baseline cardiovascular risk in healthy firefighters and compare this to an age- and sex- matched group with similar occupational responsibilities. Secondly, I wish to understand the occupational risk factors that confer an increase in the risk of on-duty cardiovascular events amongst firefighters. By designing a series of controlled and real-life exposure studies, I will be able to comprehensively assess the occupational risk factors that are likely to pose the most threat to cardiovascular health of firefighters. The following hypotheses will be addressed:

i) Off-duty firefighters do not have impaired endothelial vasomotor and fibrinolytic function or increased thrombus compared to matched controls (Chapter 3).
ii) Exposure to wood smoke at high ambient concentration will cause acute impairment of endothelial vasomotor and fibrinolytic function and promote thrombus formation in healthy firefighters (Chapter 4).
iii) Participation in fire suppression exercises will cause acute impairment of endothelial vasomotor and fibrinolytic function and promote thrombus formation in healthy firefighters (Chapter 5).
iv) Participation in active firefighting duties will cause acute impairment of vasomotor function and promote thrombus formation when compared to non-fire-fighting activities (Chapter 6).

CHAPTER TWO

METHODS

Chapter 2: METHODS

2.1 SUBJECT RECRUITMENT

2.1.1 ETHICAL CONSIDERATIONS

All studies were reviewed and approved by local research ethics committees at Umeå University, Sweden or within NHS Lothian as appropriate. All subjects were provided with written information and gave their written informed consent to participate in the studies in accordance with the Declaration of Helsinki. Further details of the ethical review process can be found at http://www.ClinicalTrials.gov/ (NCT 01813032 Chapter 3; NCT 01495325 Chapter 4; NCT 01812317 Chapter 5; NCT 01805063 Chapter 6).

2.1.2 HEALTHY VOLUNTEER FIREFIGHTERS AND POLICE OFFICERS

Healthy volunteer firefighters and police officers (aged between 18 and 60 years) were recruited by either advertisement in their place of work or on the intranet. All subjects took no regular medication (except for the oral contraceptive pill) and were non-smokers. Those with an inter-current illness were excluded and all subjects were free of the symptoms of respiratory tract infection for more than 6 weeks before participation.

Subjects participating in vascular studies attended either the Department of Respiratory Medicine and Allergy, University Hospital Umeå or the Clinical

Research Facility at the Royal Infirmary of Edinburgh for a screening visit prior to their enrolment. At this visit subjects had baseline haematology and biochemistry blood tests and a 12-lead electrocardiogram.

For the woodsmoke exposure study, standard lung function tests, comprising FEV_1 (forced expiratory volume in 1 second), FVC (forced vital capacity) and slow vital capacity, were also performed at screening. Subjects also performed a standardised cardiopulmonary exercise stress test on a bicycle ergometer to determine the exercise intensity required to maintain an average ventilation rate of 20 L/min/m^2. The determined workload was then used during the subjects' exposures to produce equivalent exposure in all.

Subjects were instructed to abstain from alcoholic beverages for the 24 hours prior to attendance for all their visits, and from caffeinated drinks for at least 4 hours. All subjects were then fasted for at least 4 hours prior to performing the vascular studies.

2.2 EXPOSURES

2.2.1 WOODSMOKE EXPOSURE

Exposures were performed in a purpose-built exposure chamber in Umeå, Sweden (***Figure 2.1***). During each exposure, subjects performed moderate exercise (to generate an average minute ventilation of 20 L/min/m^2) on a bicycle ergometer that was alternated with rest at 15-min intervals.

Wood smoke was generated using a common Nordic wood stove using birch wood in an incomplete combustion firing procedure (partial air-starved conditions), generating a soot-rich aerosol emission. To generate relatively constant incomplete combustion conditions during the 1 hour exposures, small batches (0.5-1.0 kg) birch wood logs were inserted every 5-15 min to maintain a high burn rate with repeated air-starved conditions. This procedure was in accordance with our previous wood smoke exposure study (Unosson et al., 2013). The birch wood was stored outdoors under roof cover for approximately 2.5 years before use and had a moisture content of 16-17% at the time of this study. The wood smoke was diluted with HEPA filtered air in three steps and continuously fed into and through a controlled environment exposure chamber (17 m^3) to achieve a steady state concentration.

The atmosphere in the chamber was monitored for pollutants using continuous measurement of oxides of nitrogen (NOx) (chemiluminescence, CLD 700 Ecophysics, >0.001 ppm) and carbon monoxide (CO) (IR, UNOR6N Maihak). Fine (<1 μm) particulate matter (PM_1) mass concentration was measured on-line using a tapered element oscillating microbalance (TEOM 1400, Thermo Scientific). Integrated with the TEOM a filter (Teflon) sampling line was used to determine the mass concentration gravimetrically. A CO alarm instrument (MC400, Monicon Technology) was used in the chamber during the exposures. The target PM_1 concentration in the chamber was 1,000 $\mu g/m^3$.

The equivalent mobility diameter (in the range 10-600 nm) of the wood smoke particles was measured in the chamber using a scanning mobility particle sizer (SMPS) (TSI GmbH). Organic (OC) and elemental carbon (EC) were determined using thermal-optical carbon analysis (according to the EUSAAR_2 protocol). These concentrations are regularly encountered at the perimeter of forest fires (Leonard et al., 2007) and indoors when cooking with solid fuels (Albalak et al., 2001), and are below the UK workplace 8 hour average exposure limits (HSE EH40 Workplace Exposure Limits 2005). The temperature in the chamber was maintained between 21-24 ^oC with a relative humidity of 50 %.

Figure 2-1 Whole Body Exposure Chamber

Whole body exposure chamber for human exposure to wood smoke. Image kindly supplied by Dr Nick Mills.

2.2.2 FIRE SIMULATION EXPOSURES

Firefighters attended the Scottish International Fire Training Centre, Edinburgh for the fire simulation exposure. This is a specially designed facility consisting of steel shipping containers bolted together on two levels with the internal layout of a dwelling house (**Figure 2.2**). Fires were ignited simultaneously in one or two rooms of the facility 15 min prior to the fire simulation exercise starting. Internal temperature was monitored throughout the facility at 0.5, 1.0 and 1.5 m above floor level. Fire simulation exposure was undertaken as a standardised exercise for a median duration of 20 min 22 sec (range 19 min 42 sec to 21 min 6 sec). All participants undertook the same tasks in the same order. This comprised entering the facility as part of a team of four firefighters, ascending stairs whilst dragging a water-filled hose throughout the facility, locating and attempting to extinguish the fire located on the first floor, before identifying and rescuing a casualty. Casualties took the form of 80kg dummies on the ground floor who were lifted with assistance from a second firefighter and removed from the facility. The exposure was determined to be complete by the supervising instructor as soon as the firefighter stepped outside the exposure facility. The firefighter was immediately escorted to an adjacent outbuilding where post-exposure assessments were undertaken. Following the exposure, firefighters removed their PPE and SCBA. They were advised to rehydrate as they normally would following this exercise, and to measure the volume of fluid they ingested.

Weight following the exposure was corrected for the volume of fluid ingested to determine losses during fire simulation.

Figure 2-2 Fire Simulation Exposure Centre

Fire simulation exposure centre with external staircase for use by supervising instructors (top) and 80kg dummy utilised for simulated rescue (bottom).

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2.3 VASCULAR STUDIES

Vascular endothelial dysfunction, which is generally characterised by the reduced bioavailability of endothelium-derived nitric oxide, has been shown to be an independent risk factor for cardiovascular morbidity and mortality (Schächinger et al., 2000, Al Suwaidi et al., 2000). Endothelial cell dysfunction results in adaptive changes within the vasculature with loss of endothelial integrity resulting in expression of leucocyte adhesion proteins, reduced anticoagulant activity, an enhanced inflammatory response, increased permeability and platelet activation, eventually leading to the development of atherosclerotic lesions (Ross, 1999). Whilst it would be ideal to study vascular vasomotor responses within the coronary arteries, this is clearly not without risk. However, it has been demonstrated that the responses of peripheral resistance vessels are well correlated with coronary responses and can be used as an accurate and validated surrogate (Anderson et al., 1995).

The technique of venous occlusion plethysmography to measure forearm blood flow was first described by Hewlett and van Zwaluwenburg in 1909 and has been widely used since then. The use of venous occlusion plethysmography along with unilateral intra-brachial infusion of vasoactive mediators has allowed the in-depth study of vascular homeostatic mechanisms and vascular pharmacology, and is now regarded as the ‘gold-

standard' method of determining vascular vasomotor function (Wilkinson and Webb, 2001).

2.3.1 VENOUS OCCLUSION PLETHYSMOGRAPHY

Venous occlusion plethysmography is a technique of measuring blood flow by determining the change in volume of the forearm with time and can be applied to both arms simultaneously. Both arms are placed above the level of the right atrium. An occluding cuff (EC20 Rapid Cuff Inflators (D.E. Hokanson Inc., Washington, USA)), inflated to a supra-systolic blood pressure (220 mmHg), is placed around the wrist to exclude the hand – in which there are a large number of arterio-venous connections and where the blood flow is highly temperature-dependent resulting in a different physiology and pharmacology to the rest of the forearm (Wilkinson and Webb, 2001). A second cuff is placed around the upper arm and is inflated for 10 sec in every 15 sec to 40 mmHg to occlude venous outflow from the arm, then deflated. When the cuff is inflated, blood flows into the arm but does not escape, and a linear increase in forearm volume is seen. This is detected by the use of a mercury-in-silastic strain gauge placed around the widest part of the forearm – effectively measuring stretch (Benjamin et al., 1995). Analogue voltage output from an EC-4 strain gauge plethysmograph (D.E. Hokanson Inc., Washington, USA) was processed by a PowerLab® analogue to digital converter and LabChart™ v7.0.1 software (AD Instruments Ltd., UK) and

recorded onto a Dell Latitude® laptop (Dell Computers Ltd., UK). Calibration was achieved using the internal standard of the plethysmograph.

The change in volume (calculated by assuming the forearm is a truncated cone in shape, with the widest part at the strain gauge) can then be expressed as blood flow per 100 mL tissue per minute. In resting conditions, it is known that around 70 % of blood flow goes to skeletal muscle, the remaining 30 % to the skin (Cooper et al., 1955). As skin blood flow is dependent on temperature, it is important that ambient temperature is constant, and for all these studies the room was maintained at 22-24 °C.

Here, vascular studies were carried out 2-4 hours after termination of the exposure (woodsmoke, fire simulation or after a real-life exposure) or after 72 hours off duty. Previous studies have demonstrated pronounced and reproducible effects of exposure to air pollutants on vascular endothelial function by the 2-hour time point, that are still present at 6 hours, but have largely resolved by 24 hours (Mills et al., 2005).

Subjects lay supine on a bed with both arms supported in a quiet, warm room maintained at 22 to 24 °C. Heart rate and blood pressure were monitored in the non-infused arm throughout each study with a non-invasive, semi-automated oscillometric sphygmomanometer (Omron Healthcare Ltd, Japan). Forearm blood flow was measured in both arms using venous-

occlusion plethysmography during unilateral intra-brachial infusion of vasoactive mediators (***Figure 2.3***).

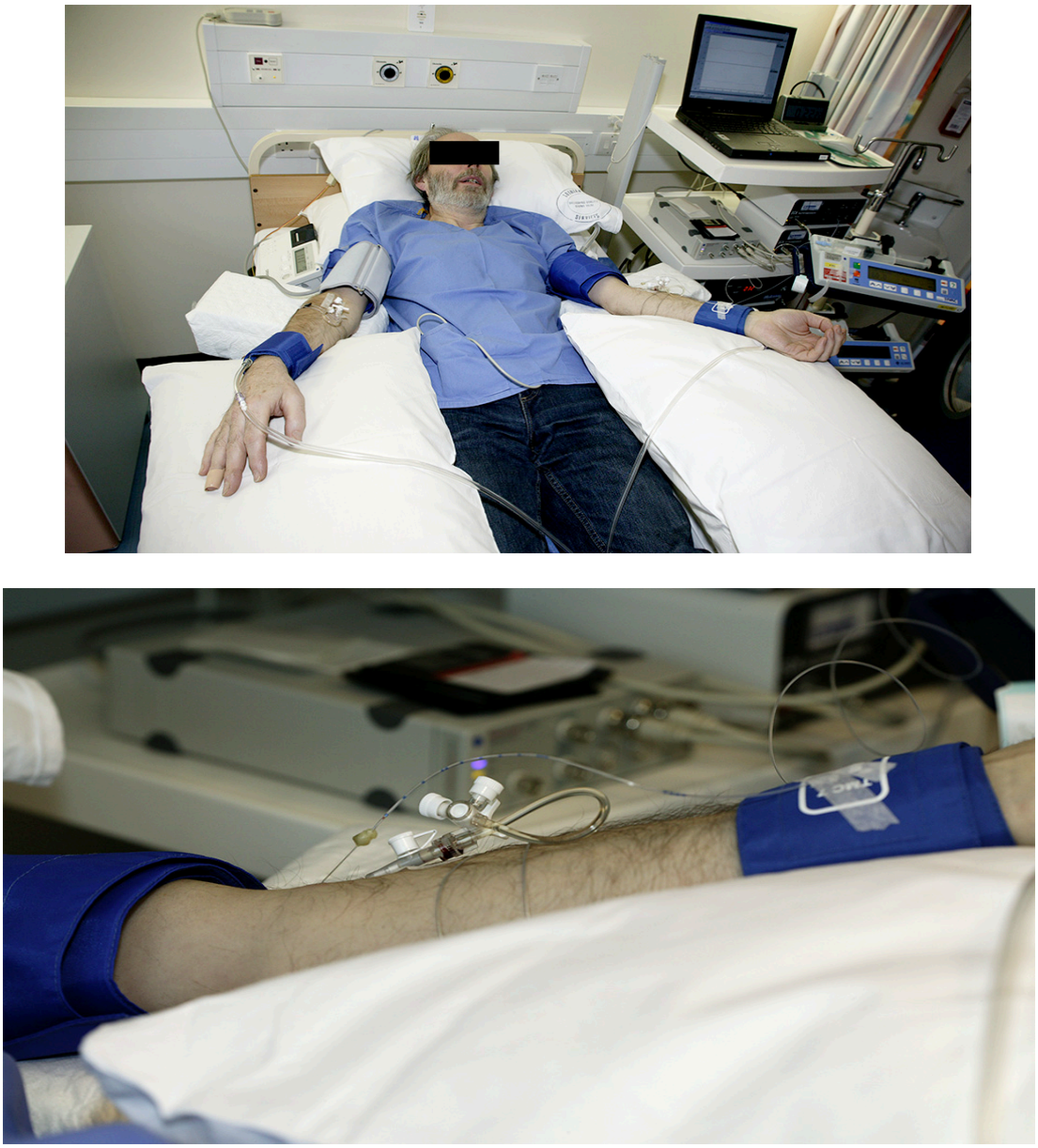
2.3.2 BRACHIAL ARTERY CANNULATION

The brachial artery of the non-dominant hand was cannulated at the start of the vascular studies using a sterile 27-standard wire gauge steel needle (Cooper Needle Works Ltd, Birmingham, UK) under aseptic technique. This needle was attached to a sterile epidural catheter and secured with sticky dental wax. This technique has been shown to be safe and simple, allowing infusion of vasoactive drugs into the forearm with minimal trauma to the vessel itself.

2.3.3 VENOUS BLOOD SAMPLING

At the start of the study, subjects had a 17-gauge intravenous cannula inserted into a large antecubital vein in each arm. This enabled venous blood sampling in both the infused and non-infused arms during the study without further venepuncture.

Figure 2-3 Forearm Venous Occlusion Plethysmography



Venous occlusion plethysmography with intra-arterial infusions of vasodilators into the brachial artery on the subjects left side along with venous occlusion cuffs (upper arm) and arterial occlusion cuffs (wrist) in a healthy volunteer (top). Arterial needle and venous cannula in-situ in the antecubital fossa (bottom).

2.3.4 INTRA-ARTERIAL VASOACTIVE MEDIATORS

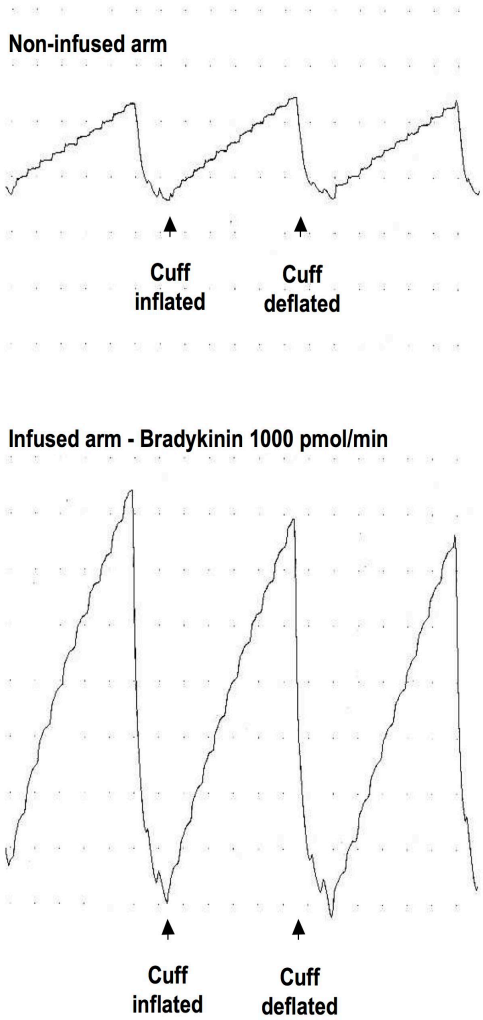
After a baseline infusion of 0.9% sodium chloride (to allow a true baseline forearm blood flow measurement to be obtained) subjects received intra-arterial infusions of acetylcholine at 5, 10, and 20 μg/min (endothelium-dependent vasodilator that does not release tissue plasminogen activator [t-PA]; Merck Biosciences); bradykinin at 100, 300, and 1000 pmol/min (endothelium-dependent vasodilator that releases t-PA; American Peptide Company); sodium nitroprusside at 2, 4, and 8 μg/min (endothelium-independent vasodilator that does not release t-PA; Hospira, Inc) and verapamil at 10, 30, and 100 μg/min (endothelium- and nitric oxide (NO)-independent vasodilator that does not release t-PA; BGP Products Ltd) were infused for 6 min at each dose. These doses are much lower than would be required to result in a systemic effect, as they need to act only within the forearm – a major advantage of this model. Subjects' blood pressure was monitored throughout the studies to assess for systemic spill over of drug using a validated, semi-automated, oscillometric sphygmomanometer (Omron Healthcare Ltd, Japan) placed around the upper part of the non-infused arm. Forearm blood flow was determined at defined timepoints during the infusion of all drugs. The 4 vasodilators were separated by 20-min saline infusions and given in a randomized order except from verapamil which was always given last due to the longer duration of action.(Robinson et al., 2006) The infusion rate was kept constant at all times at 1 mL/min.

Forearm blood flow was measured in infused and non-infused arms by venous occlusion plethysmography with a mercury-in-silicone elastomer strain gauges as described previously (Newby et al., 1997).

2.3.5 PLETHYSMOGRAPHY DATA ANALYSIS

Plethysmography data was analysed as described previously (Newby et al., 1999) by a single blinded operator. Plethysmographic data were extracted from the LabChart™ data files and forearm blood flows were calculated simultaneously by measuring the initial gradient of the curve and time in both infused and non-infused arms (***Figure 2.4***). The initial 60 seconds recordings of blood flow were not used due to variability in blood flow during this period when the cuffs are initially inflated (Benjamin et al., 1995). Ideally the final five flow recordings in each measurement period were measured. This data from LabChart™ was then imported into a template spreadsheet (Excel v16.10, Microsoft Corp, USA) where forearm blood flow was calculated and averaged for each arm. Forearm blood flow responses are reported as absolute blood flow responses (mL/100mL tissue /min) in the infused and non-infused arm where appropriate.

Figure 2-4 Forearm Blood Flow Recording



Typical forearm blood flow recording from non-infused (top) and infused (bottom) arms with infusion of intra-brachial bradykinin.

2.4 ASSESSMENT OF THROMBOSIS

Platelet-vessel wall interaction and thrombus formation is driven by blood flow rheology/haemodynamics (changes in local flow conditions), the nature of flowing blood and the characteristics of the triggering substrate (lesion type).

2.4.1 BADIMON CHAMBER

The Badimon perfusion chamber is an extracorporeal perfusion system that was developed to investigate the dynamics of platelet deposition and thrombus formation, in this case, under controlled blood flow conditions with varying degrees of stenosis, mimicking patent and stenotic coronary arteries with a substrate mimicking deep arterial injury (Badimon, 2012). The chamber provides a powerful and elegant method of assessing *ex vivo* thrombus formation and has been previously utilized to study the effects of diesel exhaust inhalation on thrombosis in man (Lucking et al., 2008, Lucking et al., 2011) and engineered medical nanoparticles on thrombosis by adding them into the extracorporeal blood flow (Raftis, 2013).

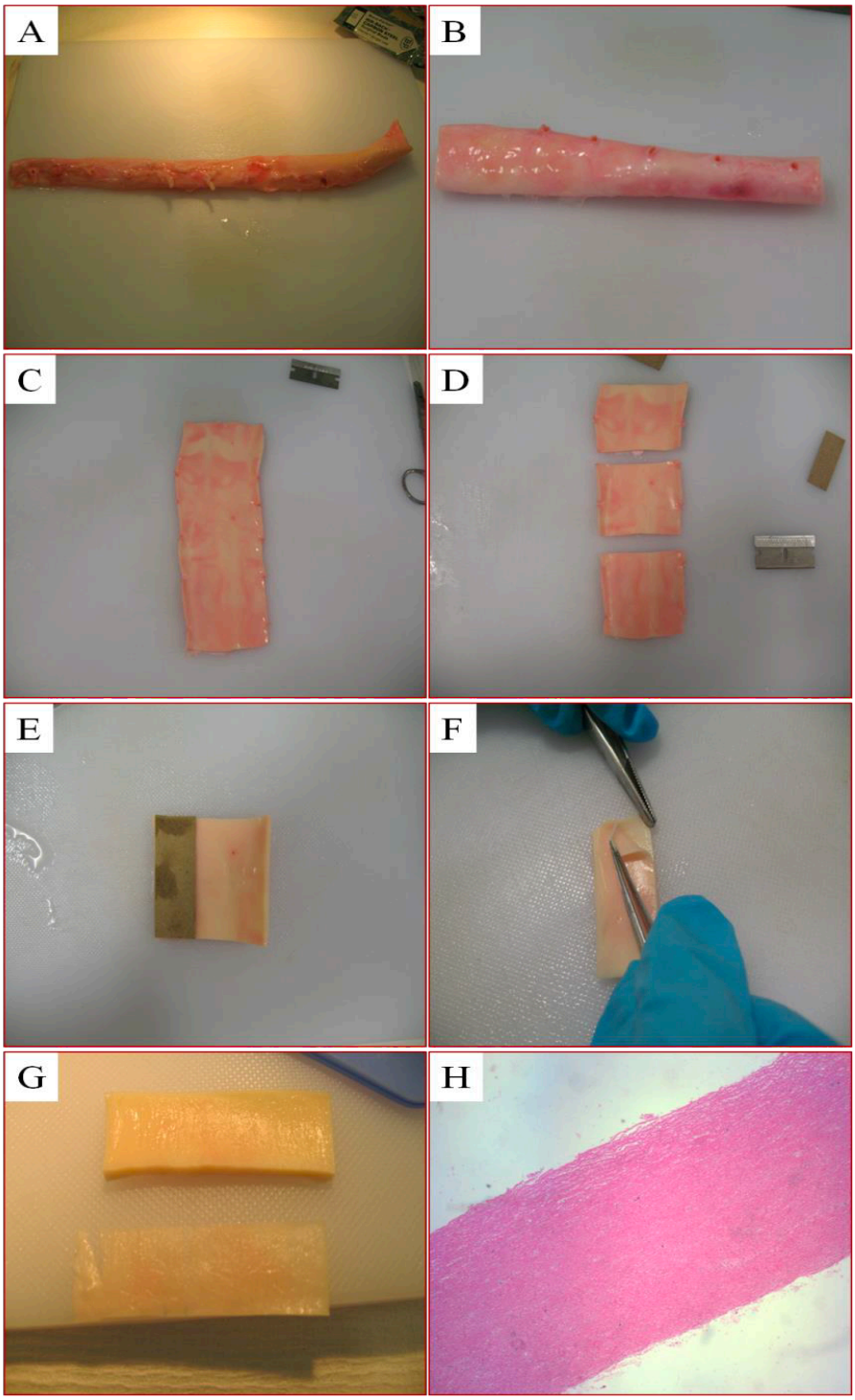
The chamber consists of a Plexiglass block through which a tubular channel of 20 or 10 mm diameter has been bored in order to mimic the tube-like shape of a blood vessel. The upper part of the plastic block is removable in order to insert, in this case, a denuded porcine arterial strip which is held in

place by the upper part which acts as a pressure plate when a screw compresses the block. The arterial strip then forms the ceiling of the tubular channel and the system is leak-free when blood is passed through the tubular channel. Three Plexiglass blocks (one 20 and two 10 mm channels) were connected in series by non-thrombogenic plastic tubes with the same diameter as the block they led in to. The rheological conditions in the first chamber simulate those of patent coronary arteries (diameter 20 mm, low-shear rate approximately 212 s^{-1}) and those in the second and third chambers simulate those of a stenosed coronary artery (diameter 10 mm, high-shear rate approximately 1690 s^{-1}). The thrombus formed in the low-shear chamber is fibrin- and macrophage-rich whereas the thrombus formed in the high shear conditions is platelet-rich (Lucking et al., 2010a). In the following studies, the Badimon chamber permitted the analysis of ex-vivo thrombus formation in response to exposures.

Porcine aortas were prepared and utilized as a vascular substrate mimicking a deep type III endothelial injury which encompasses endothelial denudation with damage to both tunica intima and tunica media and has been observed in this model to be highly thrombogenic, inducing thrombus formation at any shear rate and perfusion time. This surface, consisting of fibrillary native collagens (types III and I), proteoglycans, glycosaminoglycans, elastin, and smooth muscle cells, is exposed in a ruptured atherosclerotic wall (Badimon, 2012).

The porcine aorta is supplied in its entirety, transected proximally at the sinotubular junction and distally at the iliac bifurcation. Aortas were from male pigs whom had previously been castrated, bred for medical research and culled at one year of age (Pel-Freez® Biologicals, Arkansas, USA). The aortas were initially trimmed of perivascular adipose tissue and further transected to remove the aortic arch. The aorta was then cut into approximately 5 cm sections horizontally and then further sectioned vertically into approximately 5 x 1.5 cm strips being careful to discard sections containing origins of the major abdominal arteries or smaller branches if necessary. With the intima of the strip facing upwards the bottom right corner of the strip was removed to denote that the strip was denuded from top to bottom. Using a thin scalpel blade, strips are denuded by making a small incision in the top right corner in the tunica media then gently removing the intima and a thin layer of media by peeling it back in one single layer. The remaining tunica media was examined macroscopically for any evidence of loose tissue flaps that were subsequently removed so as not to promote the formation of further ‘false’ thrombus on the surface of the aorta which would not have otherwise formed (***Figure 2.5***, ***images A-G***).

Figure 2-5 Preparation of Porcine Aortic Strips



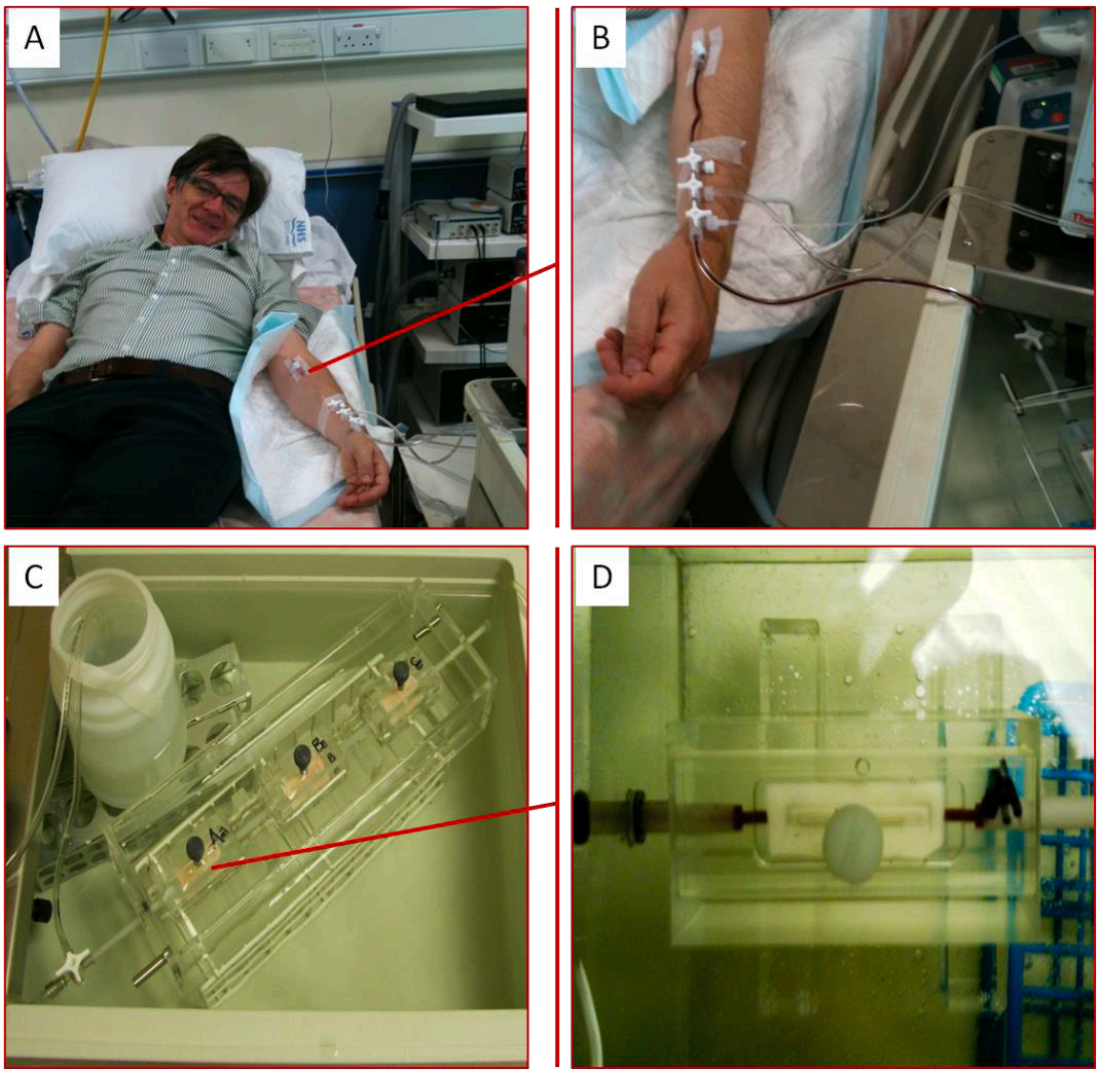
Images kindly provided by Dr Jennifer Raftis.

A 17-gauge venous cannula was inserted into a large antecubital vein. A peristaltic pump was then employed to ensure a continuous flow of venous effluent blood from the forearm (approximately 50 mL; 5-min perfusion at 10 mL/minute) that passed via a length of polyethylene tubing then through the series of plexiglass blocks which were sitting in a water bath maintained at 37 °C. Following the perfusion with blood, the chambers were flushed with 0.9% sodium chloride solution for a further one minute under the same rheological conditions to remove residual blood and non-attached cells. Following each Badimon study the porcine arterial strips were immediately fixed individually in vials of 4% paraformaldehyde for the period of 24 hours. Following this the strips were serially cut and stored in ethanol solution whilst awaiting histological preparation. The cut strips were embedded in paraffin-wax, cut into 5 μm sections, fixed on slide and stained with Masson's trichrome.

Previous studies have demonstrated that thrombus formation can be sensitively and quantitatively assessed by histological computer-assisted morphometry (Lucking et al., 2008, Lucking et al., 2010, Lucking et al., 2010, Lucking et al., 2011). With the use of a semi-automated scanning microscope (BX61, 142 Olympus, UK) and image analysis system (Ariol 3.1, Applied Imaging, USA) were used by a blinded operator to quantify thrombus area. Images were acquired at x20 magnification and total thrombus area was measured based on colour and shape high-resolution classifiers. Results

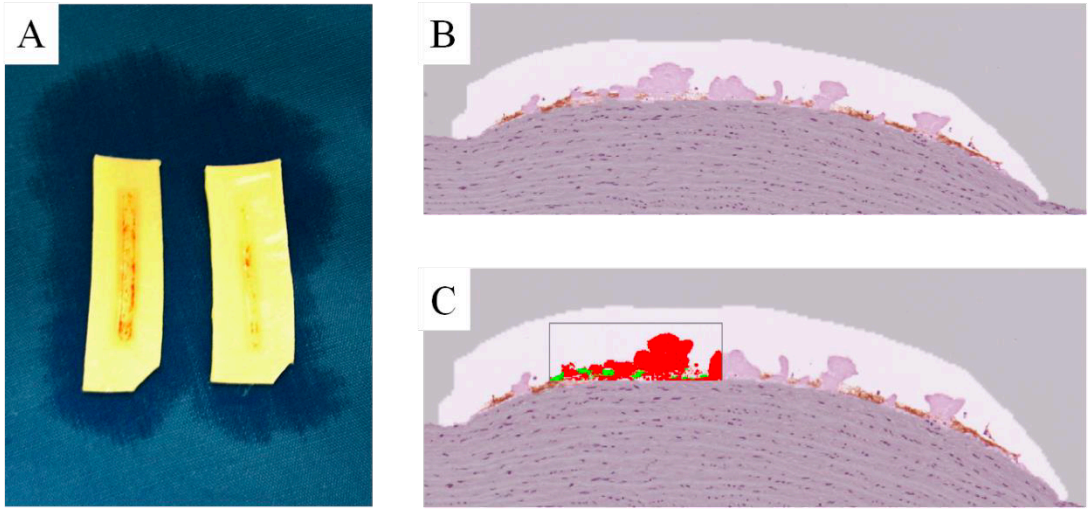
from at least six sections were averaged to determine thrombus area (μm^2) for each chamber.

Figure 2-6 Badimon Chamber Set-up with Volunteer



Images of Badimon chamber set-up showing blood flowing from subject (A-B) and through the porcine aorta lined chambers (C-D). Images kindly provided by Dr Jennifer Raftis.

Figure 2-7 Quantification of Thrombus Area



Results from at least six sections were averaged to determine thrombus area (μm^2) for each chamber. Images kindly provided by Dr Andrew Lucking.

2.4.2 FLOW CYTOMETRY

Samples were obtained and processed according to previously described protocols (Harding et al., 2007). Whole blood was obtained via a 21-gauge needle with a single and clean venepuncture of a large antecubital vein without the use of a tourniquet. Blood was drawn with a 5 mL syringe and samples were not analysed unless venesection achieved rapid and uninterrupted blood flow. The first 2 mL of blood was discarded. A further 5 mL was collected into a rohren tube containing 5 μL of the direct thrombin inhibitor, D-Phenylalanine-L-propyl-L-arginine chloromethyl ketone (PPACK, Cambridge Biosciences). The tube was gently inverted to ensure mixing and anticoagulation. Five minutes following sample collection, whole blood was then stained with the following conjugated monoclonal antibodies in three separate antibody cocktails: phycoerythrin (PE)-conjugated anti-CD14, fluorescein isothiocyanate (FITC)-conjugated anti-CD42a and allophycocyanin (APC)-conjugated anti-CD40; PE-conjugated anti-CD154, FITC-conjugated anti-CD42a and APC-conjugated anti-CD36; PE-conjugated anti-CD62P and FITC-conjugated anti-CD42a; and appropriate control isotypes (all Becton Dickinson, UK). Antibodies were diluted 1:20. Once stained, samples were incubated for 20 minutes at room temperature to identify P-selectin, CD36 and CD154 on the platelet surface and CD40 on the monocyte surface. Platelet-monocyte samples were fixed with 1.5 mL of

FACSLyse (Becton Dickinson, UK). Platelet samples were fixed with 1% paraformaldehyde.

Isotype matched controls were used to set the monocyte and platelet gates. Samples were analysed within 24 hours with Becton-Dickinson FACS Calibur flow cytometer. Platelet-monocyte aggregates were defined as monocytes positive for CD14. Platelet samples were defined as platelets positive for CD42a. Data analysis was performed using FlowJo version 7.5 (Treestar, Oregon, USA).

2.4.3 IN VIVO FIBRINOLYSIS

Fibrinolytic responses were assessed prior to and during the administration of intra-arterial bradykinin during the venous occlusion plethysmography study. Bradykinin is an endothelium-dependant vasodilator that also stimulates the release of stored tissue plasminogen activator (t-PA) from the vascular endothelium. Blood was obtained simultaneously from both 17-gauge intravenous cannulae in antecubital veins and collected in sodium citrate (PAI-1) and Stabilyte™ (t-PA) tubes on one occasion during the baseline saline intra-arterial infusion and following each dose of intra-arterial bradykinin (100, 300 and 1000 pmol/min) for analysis of t-PA and plasminogen activator inhibitor 1 (PAI-1), to assess the endogenous fibrinolytic pathway. In this forearm model, bradykinin does not directly affect

PAI-1 concentrations and, therefore, PAI-1 was only measured at baseline and at bradykinin 1000 pmol/min. Samples were immediately put on ice before being centrifuged together at 2000 G for 20 min at 4 °C. Plasma was then pipetted into three sequential aliquots for each sample and stored at -80 °C prior to analysis.

Plasma t-PA and PAI-1 antigen and activity concentrations were determined by commercially available enzyme-linked immunosorbent assays (TECHNOZYM® t-PA Combi Actibind Technoclone, Austria and Zymutest PAI-1, Hyphen Biomed, France). Intra-assay coefficients of variation were 7.0% and 5.5% for t-PA and PAI-1 antigen. All assays were performed in duplicate and the mean value taken.

A full blood count (FBC) was obtained at the beginning and end of the vascular study from the non-infused arm to measure the haematocrit and allow calculation of the net release of t-PA. Estimated net release of t-PA activity and antigen were calculated as previously described (Newby et al., 1997) with the following formula:

$$\textit{Estimated net t-PA release} = FBF \times (1-Hct) \times ([t\text{-}PA_{inf}] - [t\text{-}PA_{non}])$$

Where FBF is absolute forearm blood flow in the infused arm, Hct is haematocrit and $t\text{-}PA_{inf}$ or $t\text{-}PA_{non}$ are the concentrations of t-PA (antigen or activity) in the infused and non-infused arms respectively.

2.5 VENOUS ASSAYS

2.5.1 SAMPLE PREPARATION AND PROCESSING

Venous blood sampling was obtained for differential leucocyte count, haemoglobin concentration, platelet number and haematocrit, clinical biochemical assays, prothrombin and activated partial thromboplastin times, glucose and lactate and analysed in the regional clinical laboratories (Departments of Haematology and Clinical Biochemistry, Lothian NHS University Hospitals Trust, UK and Departments of Haematology and Clinical Biochemistry, Umeå University Hospital, Sweden).

Blood was collected in ethylene diamine tetra-acetic acid (EDTA) and lithium heparin and immediately kept on ice. Serum samples were left to clot on ice for 60 min before being centrifuged at 2000 G for 20 min at 4 °C. Serum or platelet-free plasma was then pipetted into three sequential aliquots for each sample and stored at -80°C prior to analysis.

2.5.2 HIGH SENSITIVITY CARDIAC TROPONIN I

Plasma cardiac troponin I concentrations were determined using a high sensitivity assay (ARCHITECT$_{STAT}$, Abbott Diagnostics). This assay has a limit of detection of 1.2 ng/L, an upper reference limit (99th centile) of 34 ng/L in men and 16 ng/L in women (Shah et al., 2015). All assays were performed in duplicate and the mean value taken.

2.6 MONITORING

2.6.1 BLOOD PRESSURE MONITORING AND ANALYSIS

Subjects were fitted with an ambulatory blood pressure monitor (Spacelabs 90217, Spacelabs, UK) at least 30 minutes before exposure and wore for the period of exposure and up to 24 hours thereafter depending on the study. During controlled exposures this recorded blood pressure every 15 min, otherwise it was set up to measure every 30 min during the day (07.00 to 22.00) and hourly overnight (22.00 to 07.00). Data were stored and analysed using Spacelabs Healthcare proprietary software

2.6.2 ELECTROCARDIOGRAPHIC MONITORING AND ANALYSIS

Subjects were fitted with a portable 12-lead electrocardiograph (ECG) (Lifecard CF, Delmar Reynolds Medical Ltd, UK) at least 30 minutes before exposure and wore for the period of exposure and up to 24 hours thereafter depending on the study.

Electrographic traces were analysed with the use of the Medical Pathfinder Digital 700 Series Analysis System (Delmar Reynolds Medical Ltd, UK) as previously described (Mills et al., 2007). A single operator, blinded to both subject characteristics and exposure, verified any abnormal rhythms and manually edited artefact and aberrant beats. The 30 min period of rest immediately after fitting with ECG was used to normalize the ST-segment for

each lead. Thereafter, ST-segment deviation was calculated by comparing the ST-segment amplitude during the exposure period and then across the subsequent 23-h period. The ST-segment amplitude was determined at the J-point plus 50 msec. 0.5mm events were defined as any episode with ≥0.5mm horizontal or downsloping ST-segment depression lasting at least 1 min and separated by the next event by 1 min. The ischaemic burden during each exposure was calculated as the product of the change in the ST-segment amplitude and the duration of the exposure. Leads II, V_2 and V_5 were selected *a priori* for ST-segment analysis to reflect separate regions of the myocardium. The maximum ST-segment depression and ischemic burden were determined for each lead and as a composite (Mills et al., 2007).

2.6.3 CORE BODY TEMPERATURE MONITORING

Ingestible temperature monitors were utilized together with externally worn temperature loggers (both CorTemp, HQInc) to measure core body temperature. Subjects were instructed to swallow ingestible temperature monitors at pre-specified time prior to exposure. In cases where the monitor was no longer detectable in the gastrointestinal tract at the time of study, a second monitor was swallowed at least 30 min prior to study.

2.7 CARDIOPULMONARY EXERCISE TESTING

Participants performed a continuous incremental cycling test to volitional exhaustion on a bicycle ergometer (ergoselect 200, ergoline GmbH, Germany) to determine peak VO_2 via an automated gas analysis system (Jaeger™ Oxycon Pro™, Carefusion, Germany). Each participant completed a 5-min warm-up maintaining a speed above 60 r/min with no applied resistance. The participant then had to maintain a cadence of 70 r/min or above throughout, with an increase in load by 25 Watt every 1 min until volitional exhaustion or they could no longer maintain the required cadence. Oxygen consumption (VO_2) was measured breath by breath. Peak VO_2 was defined as the highest 30 sec average recorded during the test. 12-lead ECG, blood pressure, oxygen saturations and respiratory rate were measured throughout. Verbal encouragement was given throughout. Cardiopulmonary exercise tests were performed in the Respiratory Laboratory at the Royal Infirmary of Edinburgh by trained respiratory physiologists.

2.8 DATA ANALYSIS AND STATISTICS

A sample size of 20, based on power calculations from previous studies, gives greater than 90 % power to detect a 22 % difference in forearm blood flow, a 17 % difference in mean t-PA release and 10 % difference in thrombus area at a significance level of 5 % (Newby et al., 1998, Newby et

al., 1999, Mills et al., 2005, Lucking et al., 2010). Continuous variables are reported as mean ± standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism version 5.0 (Graph Pad Software, USA) by 2-way analysis of variance (ANOVA) with repeated measures including time and exposure as variables where appropriate; and 2-tailed Student *t*-test, or Wilcoxon signed-rank where appropriate. Statistical significance was taken at two-sided $P<0.05$.

CHAPTER 3

COMPARISON OF VASCULAR FUNCTION AND CARDIOVASCULAR RISK IN EMERGENCY SERVICE PROFESSIONALS

Chapter 3: COMPARISON OF VASCULAR FUNCTION AND CARDIOVASCULAR RISK IN EMERGENCY SERVICE PROFESSIONALS

3.1 SUMMARY

Firefighters are at high-risk of cardiovascular death on-duty compared to other occupations, with coronary heart disease accounting for 45 % of deaths amongst firefighters, 22 % of deaths in police officers and 15 % of all deaths at work. We compare traditional cardiovascular risk factors, vascular endothelial function and thrombogenicity in firefighters and a matched group of police officers with similar occupational responsibilities.

Twenty healthy, non-smoking firefighters and twenty matched police officers (age 41±2 years, 17 males) were randomly selected for cardiovascular risk assessment including cardiopulmonary fitness testing. All volunteers underwent assessments whilst off-duty. Following each exposure, *ex vivo* thrombus formation, fibrinolysis, and forearm blood flow in response to intra-arterial infusions of endothelial-dependent and -independent vasodilators were measured.

Firefighters and police officers had similar body mass index (26±0.7 *versus* 28±0.7 kg/m^2, P=0.09) but firefighters had significantly higher levels of

cardiopulmonary fitness (VO_2 max 41±3 *versus* 33±2 mL/kg/m^2, P=0.03). Blood pressure, resting heart rate, lipid concentrations and QRISK®2-2017 Score (2.7±0.6 % *versus* 2.5±0.5 %) were similar between both groups (P>0.05 for all). There was a dose-dependent increase in forearm blood flow with each vasodilator (P<0.01 for all), with no difference in response to bradykinin (P=0.97), acetylcholine (P=0.85), sodium nitroprusside (P=0.84) or verapamil (P=0.77) between groups. Thrombus formation was similar in firefighters and police officers in both the low shear chamber (thrombus area 7,951 μm^2 *versus* 9,252 μm^2; P = 0.26) and high shear chamber (thrombus area 9,998 μm^2 *versus* 8,679 μm^2; P = 0.36).

Firefighters and police officers have similarly low risk of future cardiovascular event and neither have impairment of vasomotor endothelial function or increased thrombogenicity whilst off-duty. We suggest that the excess of cardiovascular events and deaths on-duty in firefighters are due to the acute and transient effects of extreme physical exertion, psychological stress, heat and exposure to air pollutants.

3.2 INTRODUCTION

In comparison to other professions with similar responsibilities including emergency call-outs, physical exertion, and dangerous duties, firefighters have an abnormally high incidence of on-duty cardiovascular death. Coronary heart disease accounts for 45% of deaths amongst firefighters, compared with 22% of deaths in on-duty police officers, 11 % in on-call medical and paramedical staff and 15% of all deaths at work (Franke et al., 2002). These statistics are even more concerning when we consider these emergency responders are subjected to rigorous medical and physical exclusion criteria at entry level and should therefore be healthier than the general population. However, the physical exclusion criteria are based on physical fitness, and cardiovascular health does not necessarily play a dominant role in defining fitness, especially over the duration of a career.

In the largest analysis of the cause of death amongst on duty firefighters performed to date, 1,144 deaths over a 10 year period from 17 large metropolitan fire departments in the United States were examined (Kales et al., 2007). Deaths were classified according to the duty performed during the onset of symptoms or immediately prior to any sudden death. This analysis found that 32% of cardiovascular deaths occurred during fire suppression, despite the fact that this activity only accounted for 1-5% of the average firefighter's professional time annually. Death from cardiovascular disease

was 12- to 136-times more likely to occur during fire suppression as during non-emergency duties. However, there was an increase in the risk of death associated with other specific emergency duties including alarm response (odds ratio 2.8 to 14.1), alarm return (odds ratio 2.2 to 10.5) and physical exertion (odds ratio 2.9 to 6.6).

Cardiovascular events in on-duty emergency health professionals are not only dangerous for the affected person but can potentially jeopardise the safety of co-workers in a close-knit team and also public safety when they are rendered incapacitated. Although both professions share some occupational responsibilities, the differences in on-duty mortality between firefighters and police officers can perhaps be explained by the unique work environment to which firefighters are often exposed. It is unclear if firefighters possess an increased baseline risk of cardiovascular events or if the risk is purely related to occupational exposures. Our aim was to undertake a comprehensive assessment of the cardiovascular health in both firefighters and police officers to assess baseline cardiovascular risk off-duty.

3.3 METHODS

3.3.1 STUDY PARTICIPANTS

Twenty healthy non-smoking firefighters were initially enrolled into the study. Twenty healthy non-smoking police officers were thereafter enrolled as age- and sex- matched controls. The study was performed in accordance with the Declaration of Helsinki, with the approval of the local research ethics committee and the written informed consent of all volunteers. Firefighters were recruited by sending study information sheets and letters to randomly selected firefighters from the Scottish Fire and Rescue Service. Police officers were then recruited after responding to an approved recruitment poster sent by mass internal email from the occupational health department of Police Scotland. Exclusion criteria included cigarette smoking, known cardiovascular disease, arrhythmias, diabetes mellitus, hypertension, asthma, use of regular medication, renal or hepatic impairment, or an inter-current infective illness. Subjects reported no symptoms of respiratory tract infection within the 4-week period preceding the study.

3.3.2 STUDY DESIGN

Subjects attended on two occasions in a case-control design. The first visit participants underwent screening and a cardiopulmonary exercise test and on the second visit comprised the main study. Subjects attended following a

period of 48 hrs off-duty to minimise the impact of confounding from other occupational exposures.

Cardiovascular assessments were performed in a quiet, temperature-controlled room maintained at 22°C to 24°C with subjects lying supine. All subjects abstained from alcohol for 24 h and from food, tobacco, and caffeine-containing drinks for at least 4 h before each vascular study. Female subjects were assessed at the same time point of their menstrual cycle.

The primary endpoints were *ex vivo* thrombus formation, forearm blood flow, and net tissue plasminogen activator (t-PA) release. Secondary endpoints were cardiopulmonary fitness levels ($VO_{2\ Max}$), fasting blood glucose, fasting cholesterol profile and mean 24h blood pressure.

Based on previous studies (Mills et al., 2005, Lucking et al., 2008, Lucking et al., 2011, Langrish et al., 2010), *ex vivo* thrombus formation was assessed 1-2 h from the beginning of the study, and forearm blood flow and t-PA release were assessed 2-4 h from the beginning of the study. Blood samples were obtained at baseline only (***Figure 3.1***).

Venous blood was analyzed for total cells, differential cell counts, and platelets by an auto-analyzer and for plasma urea and electrolytes, lipid

profile, and glucose concentration in the regional laboratories at the Royal Infirmary of Edinburgh.

Participants performed a continuous incremental cycling test to volitional exhaustion on a bicycle ergometer (ergoselect 200, ergoline GmbH, Germany) to determine peak VO_2 via an automated gas analysis system (Jaeger™ Oxycon Pro™, Carefusion, Germany) as described in Chapter 2.

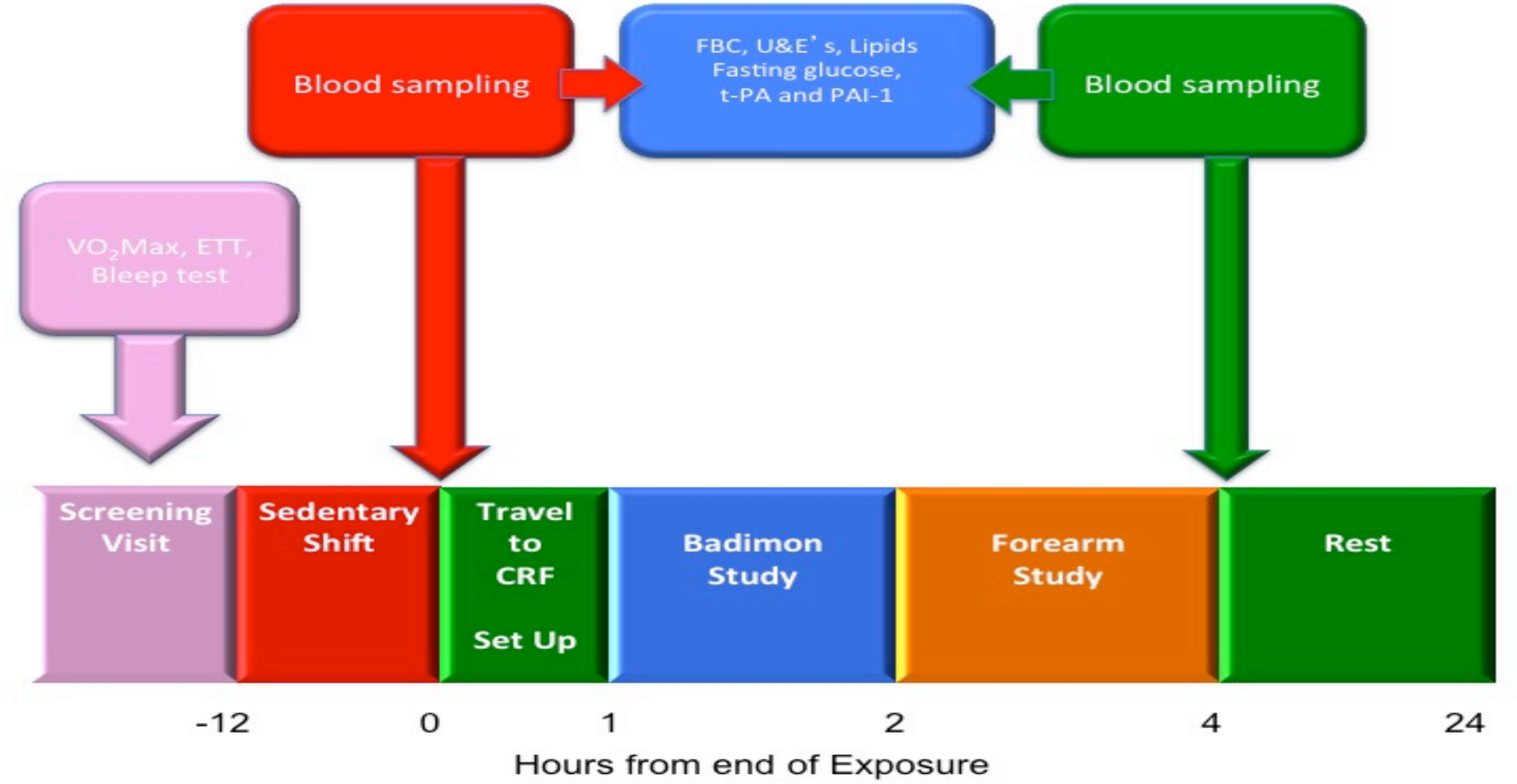


Figure 3-1 Study Design

3.3.3 EX-VIVO THROMBUS STUDIES

Thrombus formation was measured using the Badimon chamber as described previously in Chapter 2.

3.3.4 VASOMOTOR AND FIBRINOLYTIC STUDIES

All subjects underwent venous occlusion plethysmography and plasma t-PA and PAI-1 antigen concentrations were determined as described previously in Chapter 2.

3.3.5 DATA ANALYSIS AND STATISTICS

Continuous variables are reported as mean±standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism, version 5.0 (Graph Pad Software, USA) by 2-way analysis of variance (ANOVA) with repeated measures and 2-tailed Student's paired *t*-test, or Wilcoxon signed-rank as appropriate. Statistical significance was taken at two-sided $P<0.05$.

3.4 RESULTS

Twenty healthy non-smoking firefighters and twenty healthy non-smoking police officers (mean age 41±2 years, 17 males for both groups) were enrolled (***Table 3.1***). Two participants were excluded following screening visits; one police officer was found to have diabetes mellitus and one firefighter who was found to have familial hypercholesterolaemia. Both were withdrawn and referred for treatment.

3.4.1 CARDIOVASCULAR RISK PROFILES

Firefighters and police officers had similar body mass index (26±0.7 *versus* 28±0.7 kg/m^2, P=0.09) but firefighters had significantly higher levels of cardiopulmonary fitness (VO_2 max 41±3 *versus* 34±2 mL/kg/m^2, P=0.03, ***Table 3.1***). Systolic blood pressure, resting heart rate, and lipid concentrations were similar between groups (P>0.05 for all). However, firefighters had significantly lower diastolic blood pressure (73±2 *versus* 83±2 mmHg, P=0.02, ***Table 3.1***). Despite the superior cardiopulmonary fitness levels and lower diastolic blood pressure amongst the firefighter group, when a snapshot of 10 year cardiovascular event risk was obtained with the well-established and validated QRISK®2-2017 Score (Hippisley-Cox et al., 2008) these were similar between groups (2.7±0.6 % *versus* 2.5±0.5 %, P=0.72, ***Table 3.1***). The QRISK® Healthy Heart Age is the age at which a matched healthy person has the same 10 year QRISK®2 score. The mean QRISK®

Healthy Heart Age in both firefighter and police officer groups was similar (43±2 years *versus* 44±2 years, P=0.53, ***Table 3.1***) but was older than actual chronological age in both groups (41±2 years).

Table 3-1 Demographics

	Firefighters	Police Officers	P value
Age, years	41±2	41±2	1.0
Male Sex (%)	17	17	1.0
BMI, kg/m^2	26.5±0.7	28.1±0.7	0.09
Systolic BP, mmHg	131±3	137±3	0.21
Diastolic BP, mmHg	77±2	83±2	0.02
Heart Rate, bpm	57±2	57±1	1.0
Peak VO_2, ml/kg/min	41±3	34±2	0.03
Cholesterol:HDLc	4.3±0.4	4.2±0.3	0.90
QRISK®2 Score, %	2.7±0.6	2.5±0.5	0.72
QRISK® Healthy Heart Age, years	43±2	44±2	0.52

Values are reported as mean ± SEM; Student's paired t-test and Wilcoxon signed-rank test comparing groups

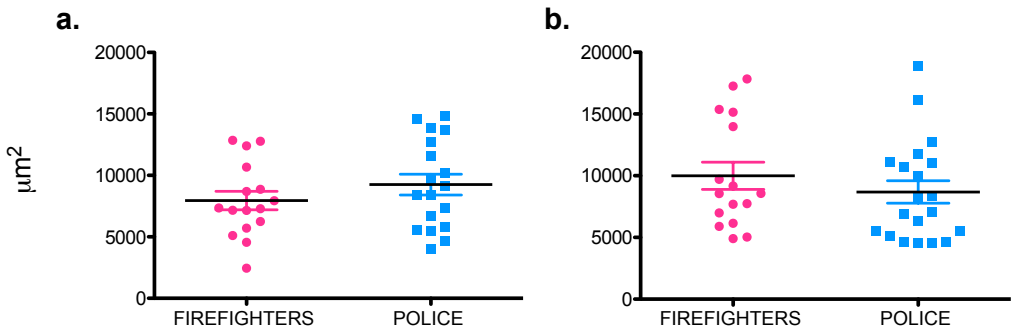
3.4.2 THROMBUS FORMATION

Thrombus formation was similar in firefighters and police officers in both the low shear chamber (thrombus area 7,951 μm^2, 95 % confidence interval [CI] 6,349 – 9,553 μm^2 *versus* thrombus area 9,252 μm^2, 95 % confidence interval [CI] 7,466 – 11,037 μm^2; P = 0.26) and high shear chamber (thrombus area 9,998 μm^2, 95 % CI 7,647-12,349 μm^2 *versus* thrombus area 8,679 μm^2, 95 % confidence interval [CI] 6,779 – 10,579 μm^2; P = 0.36, ***Figure 3.2***).

3.4.3 VASCULAR VASOMOTOR AND FIBRINOLYTIC FUNCTION

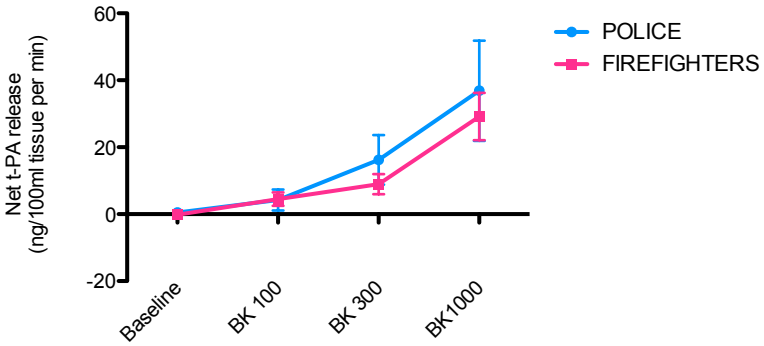
There was a dose-dependent increase in forearm blood flow with each vasodilator (P<0.01 for all). However, there were no differences in blood flow responses to acetylcholine (P=0.85), bradykinin (P=0.86), sodium nitroprusside (P=0.79) or verapamil (P=0.56) between groups (***Figure 3.3***). Bradykinin caused a dose-dependent release of tissue plasminogen activator antigen (P<0.01), which was similar after both exposures (P =0.46, ***Figure 3.4***).

Figure 3-2 Thrombus Formation



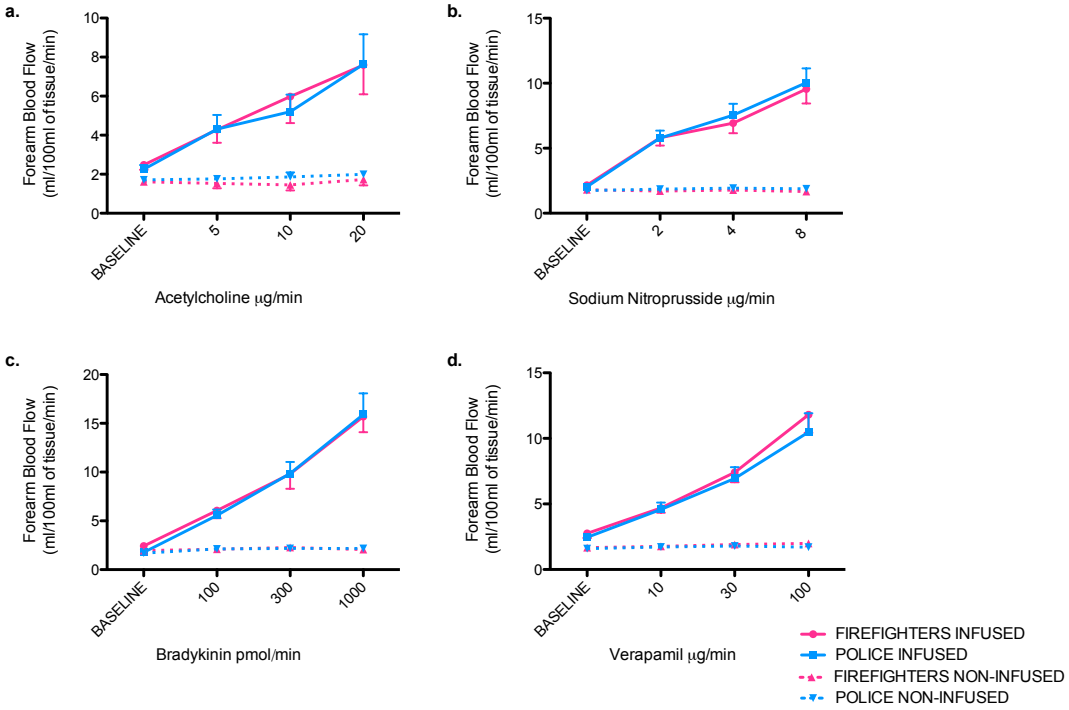
Thrombus formation under a.) low shear and b) high-shear conditions in the Badimon chamber were similar in both groups (Student's t-test, P>0.05 for both). All data expressed as mean ± SEM.

Figure 3-3 Vascular fibrinolytic function



Bradykinin caused a dose-dependent release of tissue-plasminogen activator (t-PA) antigen (2-way ANOVA with repeated measures, P<0.01), which was similar after both exposures (P=0.46). All data expressed as mean ± SEM.

Figure 3-4 Vascular vasomotor function



There was a dose-dependent increase in forearm blood flow with each vasodilator (2-way ANOVA with repeated measures, P<0.01 for all), however there were no differences in blood flow response to acetylcholine (P=0.85), bradykinin (P=0.86), sodium nitroprusside (P=0.79) or verapamil (P=0.56) between groups. All data expressed as mean ± SEM.

There are no differences in blood flow in the non-infused arms and therefore these data points are overlaid.

3.5 DISCUSSION

In a comprehensive assessment of baseline cardiovascular health, we have demonstrated that firefighters and police officers have a similar and an overall low baseline risk of future cardiovascular events. Furthermore, neither group have impairment of vascular vasomotor and fibrinolytic function nor increased thrombogenicity when off-duty. The increased risk of cardiovascular events amongst firefighters on-duty is likely therefore to be secondary to occupation-related exposures.

The majority of on-duty cardiovascular events have been noted to occur in both firefighters and police officers with recognized traditional cardiovascular risk factors (Kales et al., 2003). In previous observational studies firefighters and police officers have a prevalence of cardiovascular risk factors similar to that of the general population (Wright et al., 2011). Levels of cholesterol amongst both groups in this study would be classified as just above the normal range with a total cholesterol:HDL ratio of 4. In a large analysis of cardiovascular risk factors amongst police officers, hyperlipidaemia was prevalent in 33.2 % (Franke et al., 2002) and above 20 % of firefighters were also reported to have hyperlipidaemia (Soteriades et al., 2002, Donovan et al., 2009), Approximately three quarters of all emergency service professionals were found to have elevated blood pressure (Kales et al., 2009) whilst the presence of established hypertension is estimated to be 11-

23 % in firefighters (Choi et al., 2016, Soteriades et al., 2003) and 15-38 % in police officers (Franke et al., 2002, Violanti et al., 2009, Hartley et al., 2011). The presence of hypertension in police officers is strongly linked to on-duty cardiovascular morbidity and mortality (Pyörälä et al., 2000). In the current study, both professional groups would not be classed as having pre-existing hypertension although American guidelines just published have redefined hypertension as a blood pressure ≥ 130/70 which would reclassify both groups as mildly hypertensive (Whelton et al., 2017). Thus, both groups may be seen to benefit from lifestyle guidance and advice on levels of exertion to lower blood pressure if we are to adopt such guidelines. Blood pressure is a continuous variable with increasing levels, even within the realms of normal, associated with increasing risk of cardiovascular disease. It remains to be seen if the rest of the world will adopt the seemingly stringent stance on diagnosis of hypertension. Whilst the current blood pressure levels within both groups are acceptable based on current UK and European guidelines it is of note that the mean age of both groups in this study is only 41 years. In the Fire Service, at least, the projected age at retirement of some of our firefighter group is 60 years. Blood pressure is known to increase with advancing age so although our groups may have blood pressures within the normal range currently it would be prudent to act now to lower blood pressure if they are to remain at low cardiovascular risk in future.

In this current study, the mean body mass index (BMI) in both groups would

classify them as overweight. This is consistent with other studies where over 75 % of firefighters, police officers and ambulance personnel are classified as being overweight or obese, by BMI criteria (Franke et al., 2002, Soteriades et al., 2005). The prevalence of obesity amongst emergency professionals is of notable concern; especially when we consider the role of obesity in potentiating other cardiovascular risk factors such as type 2 diabetes mellitus and hypertension. Occupation itself may be contributing to the magnitude of the problem as weight has been demonstrated to increase with increasing years of service (Zimmerman, 2012).

Cardiovascular risk scores traditionally calculate risk as a 10-year risk estimate of a cardiovascular event such as a myocardial infarction or stroke. Contemporary cardiovascular risk scores now encompass additional risk factors such as family history and the presence of chronic kidney disease, making them more discriminatory with improved accuracy. Furthermore, contemporary risk scores also attempt to assess in terms of lifetime risk which ideally should guide a clinician's treatment of modifiable risk factors and is a more tangible concept for patients to grasp. Both professional groups in this study would be assessed as having a low risk of cardiovascular events in the coming 10 years. However, with regards to lifetime risk, both groups have an older heart age than their chronological age demonstrating that their risk profiles could be improved. Following completion of this study, I wrote to all the participants outlining their individual

risk profiles and gave personalised advice on reducing cardiovascular risk henceforth. However, a paradigm shift is required on the part of the physicians caring for these groups, most likely to be occupational health and general physicians, to recognise and treat on account of lifetime risk especially when we do not consider occupational-related risk factors in addition to traditional risk factors. Lifestyle advice and education when appropriately targeted is known to be a worthwhile intervention in reducing cardiovascular events and should be given to all employees as a matter of course, regardless of age but with particular attention paid to those over 40 years.

Contemporary cardiovascular risk scores do not encompass cardiopulmonary fitness as a parameter despite the protective role of increased levels of cardiopulmonary fitness in offsetting the risk of cardiovascular events being well established (Mittleman et al., 1993). Somewhat unsurprisingly, we demonstrated that firefighters in this study have a higher level of cardiopulmonary fitness in comparison to their police officer counterparts. The requirements for entry to both professions are similar, necessitating comparable levels of cardiovascular fitness. While UK firefighters are required to maintain a high level of cardiopulmonary fitness throughout their career, police officers and firefighters elsewhere may not have fitness levels assessed again for the duration of their career following recruitment. There is no agreed consensus on entry cardiopulmonary fitness

level in either profession but this ranges from peak VO_2 of 35-42ml/kg/min. However, the mean peak VO_2 in the firefighter group in this study was 41 ml/kg/min which is lower than the proposed standard of 42 ml/kg/min for UK firefighters regardless of age. Police officers are arguably at greater risk of cardiovascular events owing to most working full time in sedentary roles with no encouragement to achieve or maintain a high level of cardiopulmonary fitness. The onus should be on an individual to maintain a decent level of cardiopulmonary fitness however employers, in this instance the Police and Fire Services, have a responsibility to help their employees achieve and maintain fitness, especially as their occupations are associated with a higher risk of on-duty cardiovascular events. Moreover, employers have an appropriate platform to educate and promote fitness, and have the ability to effect change by making it a mandatory condition of their employment but this can only feasibly be achieved by allowing both groups to have allocated and protected exercise time at work.

Fortunately, the prevalence of most cardiovascular risk factors in our study participants are relatively low, however the presence of traditional cardiovascular risk factors is known to be higher amongst those who have had fatal cardiovascular events (Geibe et al., 2008). Furthermore, 18-26% of firefighters who experience an on –duty cardiovascular event were working with an established diagnosis of coronary heart disease, peripheral vascular or cerebrovascular disease (Kales et al., 2003, Holder et al., 2006). It is

likely, therefore, that on-duty cardiovascular events represent a complex interplay between the occupational and traditional risk factors. Moreover, preventing cardiovascular events will no doubt necessitate the modification of both occupational and traditional risk factors. The pathophysiology relating to traditional cardiovascular risk factors has been extensively researched whereas the biological mechanisms behind the occupational risk factors have not been clearly defined.

We demonstrate in this study that endothelial vasomotor and fibrinolytic function and thrombogenicity are similar in both groups at baseline following a period off-duty and can be classified as normal when compared to previous studies assessing healthy volunteers following sham exposures (Mills et al., 2005, Lucking et al., 2008, Langrish et al., 2010, Lucking et al., 2011). Endothelial dysfunction is one of the earliest pathological features of atherosclerosis (Ross, 1999) and can predict the likelihood of future cardiovascular events in at-risk individuals with normal coronary arteries (Halcox et al., 2002). While it is reassuring that both groups have no evidence of endothelial dysfunction or increased thrombogenicity at baseline, this finding lends further credence to the hypothesis that it is the occupational risk factors to which these groups are exposed that cause dynamic changes in endothelial function and thrombotic potential leading to cardiovascular events.

The physical and psychological demands imposed on both professions during emergency response duties are similar. Firefighters and police officers are often exposed to long stretches of relative inactivity, punctuated by unpredictable and stressful bursts of high intensity, and potentially life-threatening activities. The abrupt psychological stress is associated with sympathetic arousal, increases in heart rate, blood pressure and myocardial oxygen demand, stimulate inflammatory cytokines and platelet aggregation (Strike et al., 2006). Amongst firefighters, this is compounded by the lifting of heavy equipment and materials. The breathing apparatus required to be worn during fire-fighting can weigh up to 24kg and can be worn for prolonged periods. Heavy exertion particularly in individuals unaccustomed to exercise has been identified as an important trigger for sudden cardiac events (Mittleman et al., 1993) and is associated with increased oxidative metabolism and a pro-oxidant state known to be associated with endothelial dysfunction. Conversely, habitual exercise is known to improve nitric oxide mediated endothelial function and have beneficial effects on conventional risk factor profiles (Green et al., 2004).

Participation in shift work is also key to both professions and is known to disrupt normal sleep and dietary patterns (Elliot and Kuehl, 2007). Furthermore, the circadian pattern of cardiovascular death in emergency professionals is reversed and corresponds to the increased frequency of emergency call outs. Seventy seven percent of CHD deaths and 61 % of

emergency dispatches have been reported to occur between noon and midnight (Kales et al., 2003). Emergency service professionals commonly perform two 24-hour shifts per week or staggered rotating shifts, whilst many also spend days off working second jobs or overtime. Chronic sleep deprivation is therefore commonplace, which has also been linked to increased incidence of cardiovascular risk factors and cardiovascular events potentiated by increased sympathetic tone and cortisol output, creating a pro-inflammatory state and endothelial dysfunction (Wolk et al., 2005, Strike and Steptoe, 2005, Sauvet et al., 2010). Moderate-high intensity exercise has been shown to counteract the deleterious effects of sleep deprivation on endothelial function (Sauvet et al., 2017).

Exposure to heat and air pollution, may increase cardiovascular risk among firefighters. Firefighters, unlike police officers, often operate in a hostile environment and at the extremes of normal physiology. Research has identified increased heart rates and blood pressure in firefighters responding to alarms, especially when awoken from sleep, consistent with sympathetic stimulation and heart rates have often exceeded the age-predicted maximum during fire suppression (Sothmann et al., 1991, Smith et al., 1996, Smith et al., 1997, Angerer et al., 2008). Additionally, heart rates markedly exceeded the peak heart rate reached during the formal exercise test performed as part of the pre-employment medical screening. Heat stress is unique to firefighting and together with associated fluid losses result in reduced cardiac

output, systemic inflammation and a hypercoagulable state (Smith et al., 2001, Smith et al., 2001). Additionally, in contrast to police officers, firefighters are often exposed to significant air pollution. Air pollution is well–established risk factor for cardiovascular events and has been shown in previous work to be associated with impairments in endothelial function and increased thrombogenicity in healthy individuals (Mills et al., 2005, Lucking et al., 2008, Langrish et al., 2009, Miller et al., 2009, Lucking et al., 2011, Langrish et al., 2013). Substantial smoke exposure occurs during structural fires, despite the use of self-contained breathing apparatus. Whilst self-contained breathing apparatus has improved greatly and is essential when fighting internal structural fires, it does not fully eliminate pollutant exposure, and it is still commonplace to find firefighters extinguishing external fires without any respiratory protection. Moreover, respiratory protection is often abandoned during overhaul (the period immediately after fire suppression), when exposure to fine particulate matter and other toxic chemicals remain very high (Burgess et al., 2001).

In the United States, legislation recognises the occupational risk of cardiovascular disease and compensates firefighters who develop coronary heart disease on-duty (Congress, 2009). Although there is a lower rate of on-duty cardiovascular death amongst police officers, 18 US States have also passed similar legislation for cardiovascular disease in police officers (Congress, 2003). There is also provision for federal aid to the families of

police officers or other emergency service workers who die of an on-duty cardiovascular event. This type of benefit legislation creates the legal presumption that the affected worker's disease is causally related to occupational factors. Although the epidemiological data seems conclusive, there is a paucity of mechanistic data to prove conclusively that occupational factors are responsible. Much work remains to be done in this regard.

There are a few limitations to this study. Firstly, that the participants in this study are perhaps not comparable with the US firefighters and police officers who form the basis of almost all the literature about cardiovascular risk within these occupations. The participants in this study were non-smokers and were screened for hypertension and hyperlipidaemia. Therefore, they are a highly selected group. Additionally, it is often healthy subjects that volunteer for research studies as they are highly motivated and active in their approach to remaining healthy. We did try to minimise this selection bias by inviting a randomly selected group of firefighters to participate and thereafter matching them to police officers who replied to our email correspondence.

Secondly, we have calculated future cardiovascular risk based on a widely utilised and validated cardiovascular risk calculator. While this may be appropriate to give a snap shot of future cardiovascular event risk, this has not been validated specifically within these occupational groups. No risk calculator accounts for certain occupational risk factors associated with

increased rate of cardiovascular morbidity or mortality on-duty. Furthermore, it has not yet been ascertained if participation in either of these occupations confers an increased lifetime risk of cardiovascular events due to the cumulative effect of these occupational risk factors. A previous study performed coronary calcium scans on asymptomatic firefighters with a mean age of 42 years and demonstrated that firefighters have a high burden of calcified coronary atherosclerosis that was greater than anticipated on the basis of age and the presence of coronary risk factors (Santora et al., 2013). Whilst controversial, it may be advantageous, especially amongst older firefighters, to screen for underlying vascular disease especially coronary artery disease. In order to justify doing so we would need to prove that intervening reduces future events. In the SCOT-HEART study, a prospective, randomised controlled trial assessing all comers with chest pain in an outpatient setting, there was a reduction in myocardial infarction amongst those in the computed tomography coronary angiogram (CTCA) arm (SCOT-HEART Investigators, 2015). Having a CTCA led to more appropriate and targeted treatment including the initiation of preventive therapies such as statins and aspirin and was associated with a halving of fatal and non-fatal myocardial infarction (Williams et al., 2016). Until we understand more about the mechanisms underlying the increased risk of cardiovascular events in firefighters on-duty and can directly circumvent them, we arguably need to be more aggressive with our cardiovascular risk stratification.

3.6 CONCLUSION

Firefighters and police officers have similar and low risk of future cardiovascular event and neither have impairment of vasomotor endothelial function or increased thrombogenicity whilst off-duty. We suggest that the excess of cardiovascular events and deaths on-duty in firefighters are due to the acute and transient effects of extreme physical exertion, psychological stress, heat and exposure to air pollutants.

CHAPTER 4

EFFECT OF WOOD SMOKE ON VASCULAR FUNTION AND THROMBUS FORMATION IN HEALTHY FIREFIGHTERS

Extracts from this chapter have been published in

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Chapter 4: EFFECT OF WOOD SMOKE EXPOSURE ON VASCULAR FUNCTION AND THROMBUS FORMATION IN HEALTHY FIREFIGHTERS

4.1 SUMMARY

Myocardial infarction is the leading cause of death in firefighters and has been linked with exposure to air pollution and fire suppression duties. Globally, wildland firefighting comprises the single largest role of firefighters. We therefore investigated the effects of wood smoke exposure on vascular vasomotor and fibrinolytic function, and thrombus formation in healthy firefighters.

In a double-blind randomized cross-over study, 16 healthy male firefighters were exposed to wood smoke (~1 mg/m^3 particulate matter concentration) or filtered air for one hour during intermittent exercise. Arterial pressure and stiffness were measured before and immediately after exposure, and forearm blood flow was measured during intra-brachial infusion of endothelium-dependent and -independent vasodilators 4–6 hours after exposure. Thrombus formation was assessed using the *ex vivo* Badimon chamber at 2

hours, and platelet activation was measured using flow cytometry for up to 24 hours after the exposure.

Compared to filtered air, exposure to wood smoke increased blood carboxyhaemoglobin concentrations (1.3 % versus 0.8 %; $P < 0.001$), but had no effect on arterial pressure, augmentation index or pulse wave velocity ($P > 0.05$ for all). Whilst there was a dose-dependent increase in forearm blood flow with each vasodilator ($P < 0.01$ for all), there were no differences in blood flow responses to acetylcholine, sodium nitroprusside or verapamil between exposures ($P > 0.05$ for all). Following exposure to wood smoke, vasodilatation to bradykinin increased ($P = 0.003$), but there was no effect on bradykinin-induced tissue-plasminogen activator release, thrombus area or markers of platelet activation ($P > 0.05$ for all).

Wood smoke exposure does not impair vascular vasomotor or fibrinolytic function, or increase thrombus formation in firefighters. Acute cardiovascular events following fire suppression may be precipitated by exposure to other air pollutants or through other mechanisms, such as strenuous physical exertion and dehydration.

4.2 INTRODUCTION

Cardiovascular events are the leading cause of occupational death amongst firefighters and account for approximately 45 % of fatalities per year (Kales et al., 2003). Moreover, the risk of acute myocardial infarction is increased 12- to 136-fold during fire suppression duties as compared to non-emergency duties and is likely to reflect a combination of factors including extreme physical exertion, mental stress, and exposure to heat and air pollutants (Kales et al., 2007). Firefighters, during active fire suppression, are usually protected from smoke exposure by self-contained breathing apparatus (SCBA), however this is often disregarded in potentially hazardous, but tolerable situations, such as wildland fires where the long duration and remote location of firefighting often renders SCBA wearing impractical (Bolstad-Johnson et al., 2000). Respiratory protection, therefore, often takes the form of a cotton rag or bandana tied around the nose and mouth.

Air pollution is an established risk factor for the development of both acute and chronic cardiovascular diseases (Hoek et al., 2002, Miller et al., 2007, Peters et al., 2004, Langrish et al., 2012, Beelen et al., 2014, Mustafic et al., 2012, Cesaroni et al., 2014, Shah et al., 2013) with exposure to particulate matter (PM) consistently associated with adverse cardiovascular health effects. The mechanisms through which specific air pollutants, and in

particular traffic-derived air pollutants, influence the cardiovascular system have been intensively studied and an understanding of their effects on the pathophysiology of disease is emerging. In contrast, the health effects of wood smoke and biomass exposure have received little attention. Wood smoke contributes large quantities of ultrafine particles to our environment through the combustion of biomass for heating and cooking, and during major wildland fires. Firefighters have significant and often prolonged exposures during wildland fire fighting, an important duty of the fire service.

We have previously demonstrated that exposure to diesel exhaust impairs endothelial vasomotor and fibrinolytic function and increased ex-vivo thrombosis in man (Mills et al., 2005, Lucking et al., 2008). We have also demonstrated that exposure to wood smoke causes transient increases in arterial stiffness in healthy volunteers (Unosson et al., 2013). We therefore hypothesised that exposure to wood smoke, rich in ultrafine particulate matter, would have similar adverse effects and may explain the association between fire suppression and excess cardiovascular death. We therefore assessed the effect of exposure to wood smoke on vascular vasomotor and fibrinolytic function, and thrombus formation in healthy firefighters.

4.3 METHODS

4.3.1 STUDY POPULATION

Sixteen healthy non-smoking male volunteers (median age 26, range 21–26 years) were enrolled into the study. The study was performed with the approval of local research Ethics Committees, in accordance with the Declaration of Helsinki and the written informed consent of all volunteers. Firefighters were recruited using advertisements in local fire stations. Exclusion criteria were cigarette smoking or the use of snus (tobacco snuff), the use of regular medication (specifically non-steroidal anti-inflammatory drugs, vitamins or anti-oxidant supplements), known ischemic heart disease, arrhythmia, diabetes mellitus, hypertension, renal or hepatic impairment, asthma, or inter-current infection. Subjects had normal lung function and reported no respiratory symptoms in the 6-week period preceding the study. Subjects had no occupational fire exposure (wildland or structural) for a week preceding study visit.

4.3.2 STUDY DESIGN

Subjects attended on two occasions at least one week apart and were exposed to filtered air or wood smoke for one hour in a double-blind randomized crossover design. Subjects attended at 8 am on the morning of

study for initial bloods and for the fitting of Holter and ambulatory blood pressure monitors. Exposures were performed at 10 am in a dedicated exposure facility by researchers and technical staff not involved in the subsequent clinical assessment. Subjects remained indoors following exposure to minimize any confounding effects from ambient air pollution. Vascular studies were carried out in a quiet, temperature-controlled room maintained at 22 to 24 °C with subjects lying supine. All subjects abstained from alcohol and caffeine for 24 h, and from food for at least 4 h before each vascular study.

The primary endpoints were forearm blood flow, estimated t-PA release from the forearm circulation and ex-vivo thrombus formation. Secondary endpoints included arterial stiffness, platelet activation, and changes in haematological variables. Based on previous studies (Mills et al., 2005, Lucking et al., 2008, Unosson et al., 2013, Lucking et al., 2011, Langrish et al., 2010, Tornqvist et al., 2007, Lundback et al., 2009, Mills et al., 2007, Barath et al., 2010, Langrish et al., 2009, Langrish et al., 2009, Langrish et al., 2013), pulse wave analysis and velocity were assessed immediately after exposure, a study of ex-vivo thrombus formation, Badimon study was performed at 2 h, and venous occlusion plethysmography undertaken 4 to 6 h after exposures to wood smoke and filtered air. Venous blood was sampled at baseline, 2, 6 and 24 h after each exposure for storage and quantification of

carboxyhaemoglobin. Subjects were fitted with an ambulatory blood pressure monitor (Spacelabs 90217; Spacelabs, Healthcare Ltd, Hertford, UK) prior to each exposure and monitored for 24 h.

4.3.3 WOOD SMOKE EXPOSURE

Exposures were performed in a purpose-built exposure chamber in Umeå, Sweden as described previously in Chapter 2.

4.3.4 POLYCYCLIC AROMATIC HYDROCARBON ANALYSIS

The collected wood smoke PM, and polyurethane foam (PUF) plugs with sampled semi-volatile PAHs were extracted with pressurized fluid extraction using an ASE 200 Accelerated Solvent Extractor system (Dionex Corporation, Sunnyvale, CA, USA). Wood smoke PM was extracted with a solvent composition consisting of toluene and methanol 9:1 at 200 °C and 3000 psi (20.7 MPa). PUFs were extracted with hexane at 110 °C and 500 psi (3.45 MPa). Details on instrumental parameters are available elsewhere (Masala et al., 2011, Masala et al., 2014). Solid phase extraction sample clean-up was performed to remove polar constituents from the samples according to Christensen et al (Christensen et al., 2005) followed by instrumental analysis using an automated high pressure liquid chromatography-gas chromatography–mass spectrometry system (HPLC-GC-MS) (Sadiktsis et al., 2014).The HPLC part of the system was used for PAH fraction using the back flush technique, where PAHs with 3 and more rings were isolated and introduced into the GC-MS system for separation and detection. The MS was operated in selected ion monitoring mode, and the PAHs were identified using compound specific mass to charge ratio and relative retention time on the GC capillary column. In total 36 PAHs in the range of 178 – 302 Da were analysed (***Table 4.2***).

4.3.5 ELECTRON PARAMAGNETIC RESONANCE

To provide a measure of particle reactivity EPR was used to establish oxygen-centred free radical generation from particulates collected from exposures (Langrish et al., 2009). A 1.6 mm diameter section of Teflon filter from the carbon analysis filter line of the exposure chamber and suspended in physiological saline solution (Krebs buffer, composition in mM: 118.4 NaCl, 25 $NaHCO_3$, 11 glucose, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$) at a particle concentration of 100 µg/mL. Samples were vortexed for 1 min, followed by 30 min sonication sonication (100 % power; Fisherbrand FB11002; Fisher Scientific, Loughborough, UK). Suspensions were incubated with the spin-trap, Tempone-H (1 mM; Enzo Life Sciences, Exeter, UK), immediately before the initial measurement. Tempone-H is a highly sensitive spin-trap that shows selectivity for superoxide, forming a stable product that can be measured by EPR (Dikalov et al., 1997). The standard reference material urban dust (SRM1649a; National Institute of Standards and Technology, Gaithersburg, USA) was used as positive control particulate (note, that the results cannot be directly compared to filter particulates, as the proportion of the mass of wood smoke particulate unbound to the filter cannot be determined in the present study). Pyrogallol (100 µM) was used as a second positive control which spontaneously generates superoxide radicals in this buffer (Taylor et al., 2004). Samples were kept at 37 °C throughout and measurements were taken after 30 and 60 min by drawing 50 µL of

sample into a capillary tube (VWR International, Lutterworth, UK) and sealing with a plug of soft sealant (Cristaseal, VWR International). An X-band EPR spectrometer (Magnettech MS-200, Berlin, Germany) was used with the following parameters: microwave frequency, 9.3-9.55 Hz; microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 1500 mG; center field, 3365 G; sweep width, 50 G; sweep time, 30 s; number of passes, 1. Baseline signals from blank (non-exposed) filters were subtracted from that of filters with particulate.

4.3.6 Arterial stiffness

All measurements of arterial stiffness were performed at baseline, and at 10-min intervals after the exposure for one hour as previously described (Unosson et al., 2013). Pulse rate and blood pressure were measured using a validated semi-automated oscillometric sphygmomanometer (Boso-Medicus, Boso, Jungingen, Germany). Central arterial stiffness measured by pulse wave analysis was determined with a high-fidelity handheld tonometer (Millar Instruments, Texas, USA) at the right radial artery using the SphygmoCor™ system (AtCor Medical, Sydney, Australia). Carotid-femoral pulse wave velocity measurements were made using the Vicorder system (Skidmore Medical, UK).

4.3.7 VASCULAR STUDIES

All subjects underwent venous occlusion plethysmography and plasma t-PA and PAI-1 antigen concentrations were determined as described previously in Chapter 2.

4.3.8 FLOW CYTOMETRY

Samples were obtained at baseline, at 2 h immediately prior to the thrombosis study and at 24 h post exposure, and processed as described previously in Chapter 2.

4.3.9 EX-VIVO THROMBOSIS STUDIES

Thrombus formation was measured using the Badimon chamber as described previously in Chapter 2.

4.3.10 DATA ANALYSIS AND STATISTICS

A sample size of 16 gives us >90 % power to detect a 10 % difference in thrombus area, 17 % difference in mean t-PA release and 22 % difference in forearm blood flow at a significance level of 5 % (Mills et al., 2005, Newby et al., 1998, Newby et al., 1999, Lucking et al., 2010). Continuous variables are reported as mean ± standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism, version 5.0 (Graph Pad Software,

USA) by 2-way analysis of variance (ANOVA) with repeated measures and 2-tailed Student *t*-test, where appropriate. Statistical significance was taken at two-sided $P < 0.05$.

4.4 RESULTS

Exposures were well tolerated with no adverse symptoms reported and all subjects completed both study visits.

Within the chamber, particulate matter with an aerodynamic diameter <1 μm (PM_1) concentrations were 1,115 ± 151 μg/m^3, with nitrogen oxides (NO_x) and carbon monoxide (CO) concentrations of 0.6 ± 0.1 ppm and 16.0 ± 1.1 ppm respectively (***Table* 4.1*; Figure* 4-1**). Total PM mass was consistent with the target concentration for the study, as shown by both TEOM and filter measurements. The high EC/TC ratio illustrates the high soot content in wood smoke. The total PAH concentration in the exposure chamber was 4.3 ± 2.5 μg/m^3, of which 90 % was associated with wood smoke PM. The concentration of PM associated benzo[a]pyrene was 443 ± 302 ng/m^3. The most abundant PAH compounds in the wood smoke PM fraction, accounting for 88 ± 1% of the total analysed PAH (both PM associated and gas phase), were (in descending order): benzo[a]pyrene, chrysene, benzo[b]fluoranthene, benz[a]anthracene, benzo[e]pyrene, benzo[ghi]perylene, benzo[ghi]fluoranthene, indeno[1,2,3-cd]pyrene, pyrene, fluoranthene, benzo[k]fluoranthene and coronene (***Table* 4-2**).

The mean primary particle size was 168 nm and the number size distribution (mobility diameter) was clearly bi-modal with a lower peak around 50–80 nm and an upper peak around 150–200 nm (***Figure 4.2***). Wood smoke particulate generated superoxide free radicals in physiological saline solutions in the absence of cells or tissue. Comparing equivalent masses of particulate, the superoxide generating capacity of wood smoke particulates was greater than standard reference material urban dust or pyrogallol controls ($P<0.001$) (***Figure 4.2***).

Table 4.1 Characterization of wood smoke exposure and particulate matter

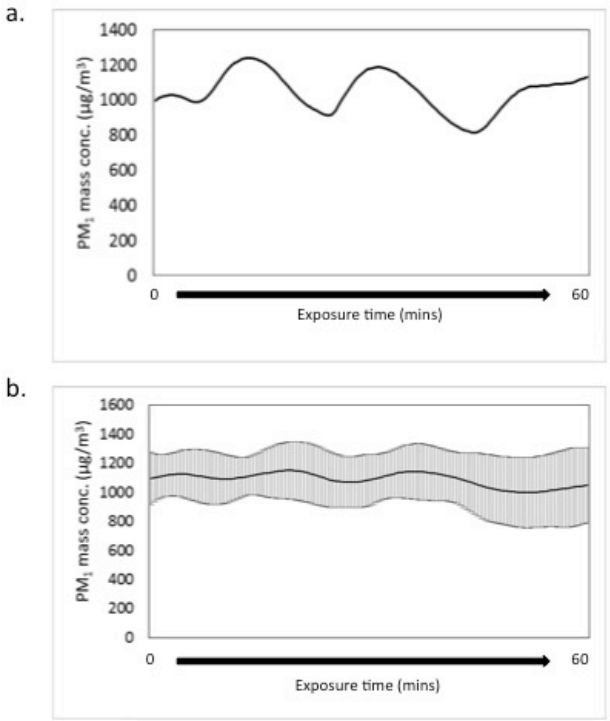
	n	Unit	Mean	SD	Min	Max
PM_1 mass concentration (TEOM)	16	µg/mg^3	1,115	151	922	1,561
PM_1 mass concentration (filter)	16	µg/mg^3	899	100	726	1105
Carbon monoxide	16	ppm	16	6	8	25
Nitrogen oxides	16	ppm	0.6	0.3	0.3	1.0
Elemental carbon/total carbon[1]	6	ratio	0.80	0.02	0.79	0.83
Organic fraction of total PM[1,2]	6	%	23.1	4.7	16.9	28.8
Soot fraction of total PM[1]	6	%	60.1	15.7	39.9	78.8
PAH – PM associated (filter)[1,3]**	6	µg/m^3	3.9	2.3	1.5	6.7
PAH – semi-volatile (PUF)[1,3]	6	µg/m^3	0.4	0.3	0.1	0.9

[1] OC-EC and PAH analysis from selected samples throughout the campaign (n = 6).

[2] Estimated based on the OC-EC analysis (assuming a factor of 1.8 used to covert OC to total organic PM and a factor of 1.1 used to convert EC to total soot PM).

*[3] Includes the PAHs: phenanthrene, anthracene, 4*H*-cyclopenta[def]phenanthrene, 2-phenylnaphthalene, fluoranthene, pyrene, 1-methylfluoranthene, benz[a]fluorene, benz[b]fluorene, 2-methylpyrene, 4-methylpyrene, 1-methylpyrene, benzo[c]phenanthrene, benzo[ghi]fluoranthene, benzo[b]naphtho[1,2-d]thiophene, benz[a]anthracene, chrysene, 3-methylchrysene, 2-methylchrysene, 6-methylchrysene, 1-methylchrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, picene, benzo[ghi]perylene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, coronene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene.*

Figure 4-1. Particulate matter variability during exposures



(a*) A typical time-series of particle mass concentrations (PM_1) in the chamber during a single 1 hour exposure measured with TEOM with data points each 30 s. (b) Time-series of mean particle mass concentrations (PM_1) in the chamber during the 1 hour exposures measured with TEOM (n=16) every 30 seconds with standard deviations as error bars.*

Table 4-2. Mean concentration of polyaromatic hydrocarbons (PAH)

	PM (ng/m^3)	PUF (ng/m^3)
Benzo(a)pyrene	443	0
Chrysene	433	1
Benzo(b)fluoranthene	409	0
Benz(a)anthracene	353	1
Benzo(e)pyrene	300	0
Benzo(ghi)perylene	298	0
Benzo(ghi)fluoranthene	237	4
Indeno(1,2,3-cd)pyrene	223	0
Pyrene	204	92
Fluoranthene	201	102
Benzo(k)fluoranthene	197	0
Coronene	134	0
Benzo(c)phenanthrene	80	3
Perylene	58	0
Dibenz(a,h)anthracene	36	0
Picene	35	0
Phenanthrene	33	159
Indeno(1,2,3-cd)fluoranthene	32	0
1-Methylfluoranthene	24	2
2-Methylchrysene	23	1
1-Methylpyrene	20	3
4-Methylpyrene	17	5
Dibenzo(a,e)pyrene	16	0
6-Methylchrysene	15	1
Benz(a)fluorene	14	2
1-Methylchrysene	12	1
2-Phenylnaphthalene	10	10
Anthracene	10	19
2-Methylpyrene	10	3
3-Methylchrysene	9	1
Dibenzo(a,i)pyrene	7	0
Benz(b)fluorene	6	2
4H-cyclopenta(d,e,f)phenanthrene	3	8
Dibenzo(a,l)pyrene	2	0
Dibenzo(a,h)pyrene	2	0
Benzo(b)naphto(1,2-d)thiophene	0	0

PM: particulate matter, PUF: polyurethane foam

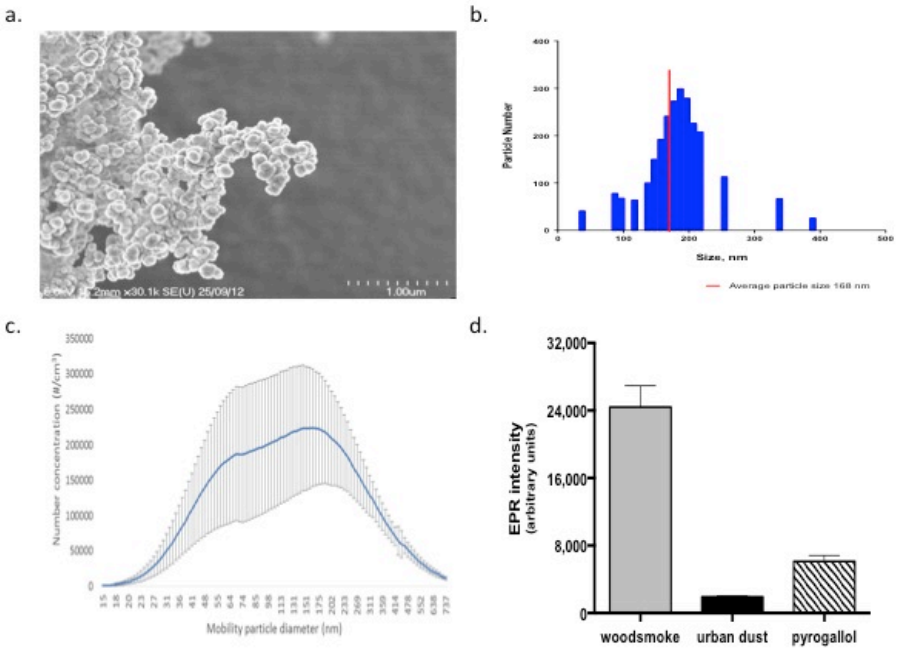
4.4.1 HAEMATOLOGY

Blood carboxyhaemoglobin concentrations increased from 0.9 ± 0.04 to 1.3 ± 0.04 % immediately following exposure to wood smoke ($P < 0.001$) (***Table*** 4-3). Total leucocyte, lymphocyte, neutrophil and platelet counts, were unaffected for up to 24 h after the exposure.

4.4.2 ARTERIAL STIFFNESS AND VASCULAR FUNCTION

Resting blood pressure and heart rate were unchanged during either exposure or for up to 24 h after exposure (***Table* 4-4**). Augmentation index, augmentation pressure and pulse wave velocity increased immediately after exposure ($P > 0.05$), but changes were similar following exposure to wood smoke and filtered air ($P > 0.05$ for all comparisons of wood smoke versus filtered air).

Figure 4-2. Characterization of wood smoke particulate matter



(a) Scanning electron microscopy (SEM) image of wood smoke particles. (b) Size distribution graph of the particle size as assessed by photon correlation spectroscopy with the mean primary particle size indicated by the red line. (c) Average particle number size distribution in the exposure chamber, measured by SMPS system. The plot displays the distribution as mean and standard deviation from all 16 exposures. Previous studies demonstrate the 50-80 nm peak consists of alkali salt particles (e.g. potassium sulphate and potassium chloride) and the 150-200 nm peak soot particles with more organic material (Londahl et al., 2008, Lamberg H, 2011). (d) Electron paramagnetic resonance (EPR) signal intensity showing oxygen free radical generation from wood smoke particulates in the presence of the superoxide-selective spin-trap Tempone-H. Particulates from exposures collected on Teflon filters suspended in physiological saline solution at a concentration of 100 µg particles/mL. The standard reference material urban dust (100 µg particles/mL) and pyrogallol (100 µM) were used as controls. Data expressed as mean ± SEM (n=4-5).

Table 4-3. Haematological effects of exposure to wood smoke and filtered air

		Filtered Air	Wood smoke	P value[1]
Carboxyhaemoglobin, %	Baseline	0.9 ± 0.0	0.9 ± 0.0	<0.001
	2 hours	0.8 ± 0.0	1.3 ± 0.0*	
	24 hours	0.8 ± 0.0	0.8 ± 0.0	
Leucocytes x 10^9 cells/L	Baseline	5.5 ± 0.4	5.6 ± 0.4	0.22
	2 hours	6.1 ± 0.3	6.4 ± 0.4	
	6 hours	6.5 ± 0.3	6.9 ± 0.4	
	24 hours	5.4 ± 0.3	5.6 ± 0.4	
Lymphocytes x 10^9	Baseline	2.0 ± 0.2	2.0 ± 0.2	0.11
cells/L	2 hours	1.9 ± 0.1	1.8 ± 0.1	
	6 hours	2.0 ± 0.1	2.1 ± 0.1	
	24 hours	2.0 ± 0.2	2.0 ± 0.2	
Neutrophils x 10^9 cells/L	Baseline	2.8 ± 0.3	2.9 ± 0.3	0.20
	2 hours	3.6 ± 0.3	3.9 ± 0.4	
	6 hours	3.8 ± 0.3	4.1 ± 0.3	
	24 hours	2.8 ± 0.2	3.0 ± 0.3	
Platelets x 10^9 cells/L	Baseline	225 ± 6	229 ± 7	0.07
	2 hours	212 ± 6	219 ± 7	
	6 hours	205 ± 5	203 ± 9	
	24 hours	225 ± 7	234 ± 6	

Values are reported as mean ± SEM;

[1] 2-way ANOVA with repeated measures comparing filtered air and wood smoke exposures

**P<0.001 for 2-way ANOVA with Bonferroni correction comparing time point with baseline*

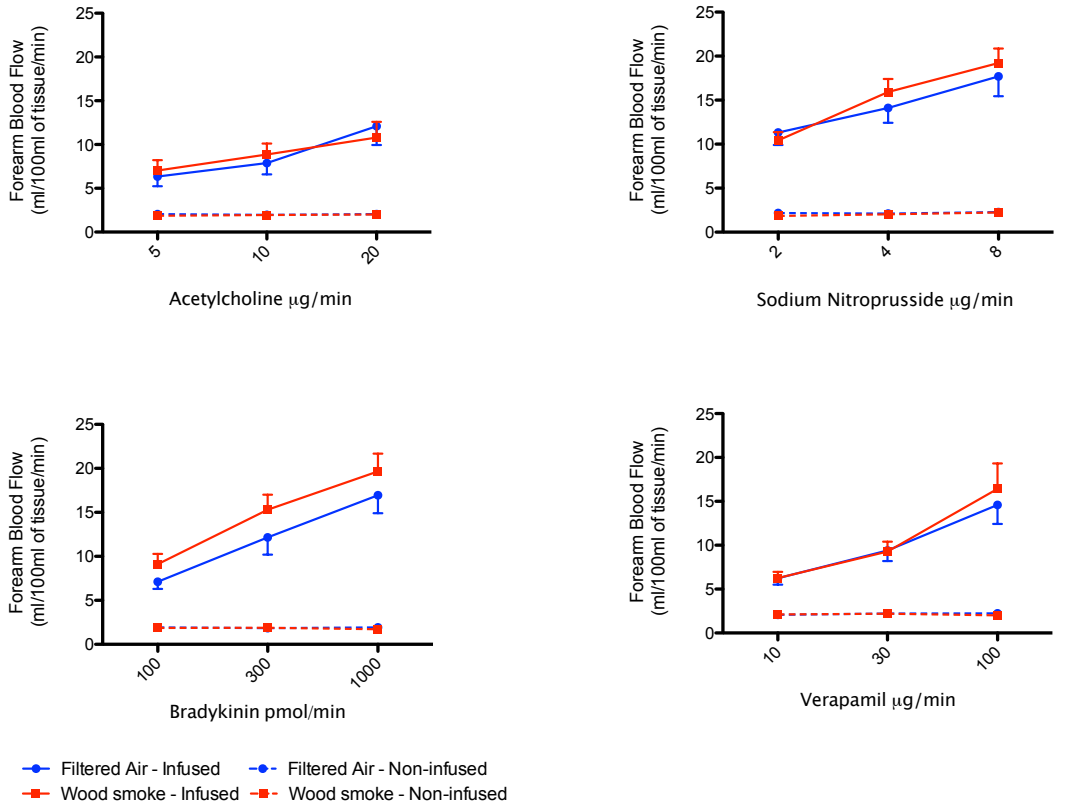
Table 4-4. Haemodynamic effects of exposure to wood smoke and filtered air

			Post-exposure										
		Baseline	0 min	10 min	20 min	30 min	40 min	50 min	1 hr	2 hrs	6 hrs	24 hrs mean	P-value[1]
Systolic pressure, mmHg	Filtered air	135 ± 2	134 ± 2	133 ± 2	130 ± 3	131 ± 2	131 ± 2	130 ± 3	133 ± 2	132 ± 2	130 ± 2	124 ± 2	0.59
	Wood smoke	132 ± 2	135 ± 3	131 ± 3	130 ± 3	131 ± 3	130 ± 3	130 ± 3	135 ± 4	134 ± 5	131 ± 3	127 ± 2	
Diastolic pressure, mmHg	Filtered air	75 ± 2	74 ± 3	75 ± 2	75 ± 2	73 ± 4	76 ± 2	76 ± 2	76 ± 2	76 ± 2	76 ±27	68 ± 2	0.89
	Wood smoke	75 ± 2	74 ± 2	74 ± 2	73 ± 2	74 ± 2	74 ± 2	76 ± 2	77 ± 2	77 ± 2	75 ± 2	70 ± 2	
Heart rate, bpm	Filtered air	63 ± 2	63 ± 3	63 ± 3	61 ± 3	60 ± 2	59 ± 2	59 ± 2	58 ± 2	58 ± 2	59 ± 2	56 ± 2	0.12
	Wood smoke	61 ± 3	63 ± 3	63 ± 3	62 ± 3	62 ± 3	61 ± 3	61 ± 3	60 ± 3	60 ± 3	58 ± 3	55 ± 2	
Δ Augmentation pressure, mmHg	Filtered air	-	0.2 ± 0.5	-0.4 ± 0.7	-0.7 ± 0.6	-0.1 ± 0.7	-0.2 ± 0.7	0.1 ± 0.7	0.7 ± 0.7	-	-	-	0.90
	Wood smoke	-	1.0 ± 0.8	-0.2 ± 0.3	-0.5 ± 0.3	-0.7 ± 0.4	-0.3 ± 0.5	0.6 ± 0.5	0.2 ± 0.5	-	-	-	
Δ Augmentation Index @75 bpm, %	Filtered air	-	0.01 ± 4.6	-1.7 ± 6.7	-3.9 ± 6.1	-2.8 ± 7.6	-3.0 ± 7.5	-2.2 ± 7.0	-1.2 ± 7.2	-	-	-	0.72
	Wood smoke	-	2.3 ± 7.6	-1.1 ± 4.0	- 2.0 ± 5.1	-3.2 ± 5.0	-3.0 ± 6.4	-1.3 ± 6.7	-1.6 ± 6.0	-	-	-	
Δ Pulse wave velocity, m/s	Filtered air	-	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	-0.1 ± 0.1	0.0 ± 0.1	0.4 ± 0.5	-	-	-	0.98
	Wood smoke	-	0.0 ± 0.1	-0.1 ± 0.1	0.0 ± 0.1	-0.1 ± 0.1	0.0 ± 0.1	-0.1 ± 0.1	0.1 ± 0.1	-	-	-	

Values are reported as mean ± SEM;

[1] 2-way ANOVA with repeated measures comparing wood smoke and filtered air

Figure 4-3. Effect of wood smoke and filtered air on forearm blood flow



There was a dose-dependent increase in forearm blood flow with each vasodilator (2-way ANOVA with repeated measures, P<0.01 for all), however there were no differences in blood flow response to acetylcholine (P=0.91), sodium nitroprusside (P=0.52) or verapamil (P=0.63) between exposures. In contrast, there was an increase in the forearm blood flow to bradykinin following exposure to wood smoke compared to filtered air (P=0.003). All data expressed as mean ± SEM.

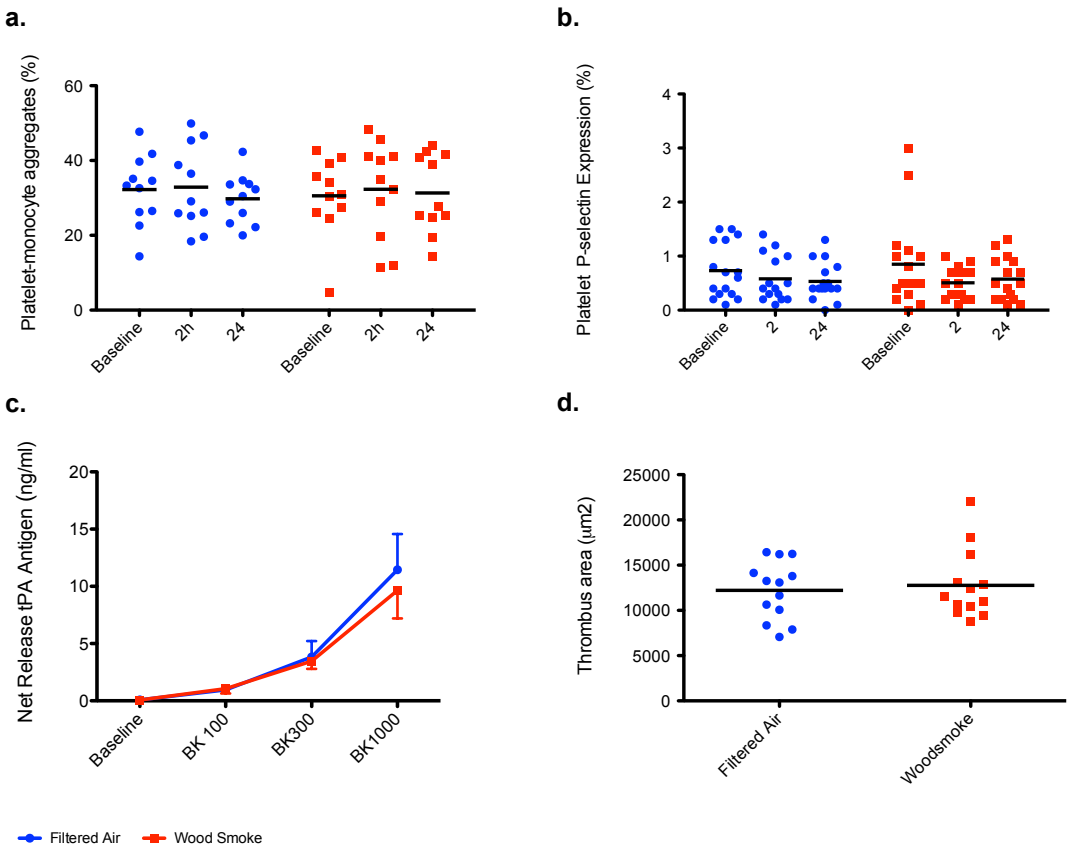
There are no differences in blood flow in the non-infused arms and therefore these data points are overlaid.

There was a dose-dependent increase in forearm blood flow with each vasodilator ($P < 0.01$ for all). However, there were no differences in blood flow responses to acetylcholine ($P = 0.91$), sodium nitroprusside ($P = 0.52$) or verapamil ($P = 0.63$) between exposures (***Figure* 4-3**). In contrast, there was an increase in forearm blood flow with bradykinin infusion following exposure to wood smoke compared to filtered air ($P = 0.003$). Bradykinin caused a dose-dependent release of tissue plasminogen activator antigen ($P < 0.01$), which was similar after both exposures ($P = 0.72$) ().

4.4.3 PLATELET ACTIVATION AND THROMBOSIS

Platelet-monocyte binding, monocyte surface expression of CD40 and platelet surface expression of CD40L and P-selectin were similar following wood smoke and filtered air exposure at 2 and 24 h following exposure ($P > 0.05$ for all) (***Figure* 4-4**). There was no difference in thrombus formation following exposure to wood smoke compared with filtered air (thrombus area 12,216 ± 3,237 *versus* 12,775 ± 3,831 μm^2, $P = 0.54$) (***Figure* 4-4**).

Figure 4-4. Effect of wood smoke and filtered air on platelet activation, fibrinolysis and thrombus formation *ex vivo*



(a) Platelet-monocyte binding and (b) platelet expression of P-selectin were unchanged 2 and 24 hours following exposure to wood smoke or filtered air (ANOVA with repeated measures, P>0.05, n=11-16). (c) Bradykinin caused a dose-dependent release of tissue-plasminogen activator (t-PA) antigen (2-way ANOVA with repeated measures, P<0.01), which was similar after both exposures (P>0.05, n=16). (d) Thrombus formation under high-sheer conditions in the Badimon chamber was similar 2 hours after exposure to wood smoke or filtered air (Student's t-test, P>0.05, n=13). All data expressed as mean ± SEM.

4.5 DISCUSSION

Controlled exposure to wood smoke at high particulate concentrations does not impair endothelial-dependent or -independent vasodilation or increase thrombosis in firefighters. Using established methodology and a comprehensive assessment of cardiovascular health we found no adverse effects of wood smoke to explain the cardiovascular risk associated with fire suppression duties.

Whilst there have been no prior controlled exposures to wood smoke in firefighters, the effect of wood smoke on vascular function has been studied in healthy volunteers (Forchhammer et al., 2012) and systemic inflammatory effects have been observed in firefighters responding to forest fires (Swiston et al., 2008, Adetona et al., 2013, Hejl et al., 2013). We have previously demonstrated that exposure to wood smoke for 3 hours at a lower PM concentration of 300 μg/m^3 caused a transient increases in arterial stiffness and heart rate (Unosson et al., 2013). In contrast, we found no effect on arterial stiffness or heart rate following exposure to wood smoke at three-fold higher concentrations in firefighters over one hour. The overall dose was similar in both studies. These discordant findings may be explained by differences in susceptibility to wood smoke between healthy volunteers and firefighters with the latter having had multiple previous exposures to wood

smoke through their occupation. There is some evidence that repeated exposure to smoke upregulates anti-oxidant levels in the airways and may diminish the effects of an acute exposure (Morrison et al., 1999). However, we restricted enrolment to those firefighters who had not attended a major structural or wildland fire for more than one week prior to study visits and the effects of previous exposure on anti-oxidant levels are likely to be transient. It is also interesting to note that the baseline heart rates in our previous study (Unosson et al., 2013) were approximately 10 bpm higher than in this study, perhaps suggesting previous participants were more susceptible to any effects of exposure on the autonomic nervous system. The duration of exposure may also be important with effects of wood smoke on arterial tone emerging after longer exposure periods. However, Forchhammer et al. recently observed no effect of wood smoke on peripheral arterial tone assessed by finger plethysmography despite delivering PM at 350 $\mu g/m^3$ for up to 3 hours (Forchhammer et al., 2012).

Venous occlusion plethysmography with intra-arterial infusion of vasodilators is widely regarded as the ‘gold-standard’ assessment of vascular function. We found no detrimental effect of exposure to wood smoke or filtered air on either endothelium-dependent or -independent vasodilatation. In fact, we demonstrate a small increase in blood flow in response to bradykinin infusion following wood smoke exposure. It is plausible this was due to the effects of

carbon monoxide exposure at concentrations that were sufficient to increase carboxyhaemoglobin concentrations and indeed, carbon monoxide is emerging as an important mediator of the vasodilator effects of bradykinin in the vessel wall (Furchgott and Jothianandan, 1991). The levels of carbon monoxide in this study were 4-fold higher than previous exposures to dilute diesel exhaust (Lucking et al., 2011) and therefore it is plausible that vasodilation as a consequence of higher gaseous pollutants (carbon monoxide or nitrogen oxides (Langrish et al., 2010), may be important here. Nevertheless, it is unlikely that this would offset any detrimental effects of wood smoke PM on forearm blood flow across other vasodilators. There were no other important or adverse effects of wood smoke on vascular function, including endogenous fibrinolysis, platelet activation and thrombosis. Taken together these findings suggest that exposure to wood smoke is unlikely to be the primary cause of acute adverse cardiovascular events in firefighters.

Whilst traffic-related air pollution is an established trigger for acute myocardial infarction (Hoek et al., 2002, Peters et al., 2004, Mustafic et al., 2012, Nawrot et al., 2011), there are few studies that have linked exposure to wood smoke or biomass with cardiovascular events. The risk in firefighters may be mediated by other factors, such as exposure to extreme heat, physical exertion and psychological stress. Heat stress results from both high

ambient temperatures and exercise-induced metabolic activity, exacerbated by insulated protective clothing. In controlled studies, heat stress causes vasodilatation and fluid loss, resulting in a reduction in cardiac output and a hypercoagulable state.(Smith et al., 2001, Angerer et al., 2008, Smith et al., 2014, Smith et al., 2011) Strenuous physical exertion is an independent trigger of sudden cardiovascular events, particularly in individuals unaccustomed to exercise (Mittleman et al., 1993).

Fire suppression often requires firefighters to work at the extremes of physical capability associated with heart rates in excess of age predicted maximums (Smith et al., 2001, Angerer et al., 2008, Manning and Griggs, 1983, Sothmann et al., 1992, Del Sal et al., 2009) and for long periods with shifts frequently lasting 12 to 24 h (Naeher et al., 2007). Whilst exposure to wood smoke may not in isolation cause vascular dysfunction or induce a prothrombotic state, it remains plausible that firefighters responding to wildland fires are at increased risk of an acute cardiovascular event through a combination of factors that could still include wood smoke. Furthermore, firefighters are also exposed to a heterogenous mix of air pollutants during other activities and although the use of breathing apparatus is employed in these situations, at the perimeter of such fires and in the aftermath when, breathing apparatus is removed important exposures may occur.

It is perhaps surprising that exposure to fine wood smoke particles at concentrations in excess of 1,000 μg/m^3 had no adverse effects given that exposure to diesel exhaust at 300 μg/m^3 has previously been shown to impair vascular function and increase thrombus formation in healthy men (Mills et al., 2005, Lucking et al., 2008, Tornqvist et al., 2007). Differences in particle properties such as size, composition and surface chemistry between these exposures are likely to be important. Although the majority of wood smoke and diesel exhaust particles are in the ultrafine size fraction, the diameter of primary wood smoke particles was 5-fold larger than diesel exhaust particles (primary particle size of NIST standard reference material 2975 is 31 nm). Wood smoke particles are therefore perhaps less likely to deposit in terminal bronchioles or alveolar space and therefore to translocate or deliver soluble components into the circulation where they could directly effect the cardiovascular system. If wood smoke particles are unable to translocate due to larger size then they could perhaps cause a systemic inflammatory response, exerting late effects that were missed by undertaking assessments early after exposure. Others have shown that controlled exposure to fine and coarse PM is associated with early autonomic imbalance: rapid elevation of blood pressure and heart rate, and decreased heart rate variability immediately following exposure (Brook et al., 2014, Devlin et al., 2003, Gold et al., 2000, Pope et al., 1999). Conversely, we may have missed any immediate effects mediated by autonomic imbalance that were not comprehensively assessed in this study, although blood pressure

and heart rate were unaffected acutely and over the 24 hour study period. Differences in surface chemistry are also likely to be important. Whilst wood smoke particles were able to generate superoxide radicals, there were major differences in the PAH profile between wood smoke particles, where high molecular weight PAHs (≥228 Da) dominated, as compared to diesel exhaust particles (Sadiktsis, 2014).

There are some limitations to our study that merit consideration. The time points chosen to conduct our assessments post-exposure were based on the results of previous studies (Mills et al., 2005, Lucking et al., 2008, Unosson et al., 2013, Lucking et al., 2011, Langrish et al., 2010, Tornqvist et al., 2007, Lundback et al., 2009, Mills et al., 2007, Barath et al., 2010, Langrish et al., 2009, Langrish et al., 2009, Langrish et al., 2013). However, it is possible that wood smoke particles either exert an immediate or late effect on the cardiovascular system and we may have missed such effects. Additionally, the duration of exposure is also likely to be important with cumulative exposures over many days or weeks difficult to model experimentally. We recruited early career firefighters to minimize potential for confounding due to pre-existing vascular disease. Firefighters are exposed to complex mixtures of air pollutants derived from different sources, many of which may be more toxic than our simulated wildland fire exposure. It is plausible that firefighters with risk factors or subclinical disease would be more susceptible to any

adverse cardiovascular effects of wood smoke. According to the widely recognized “healthy worker effect” in occupational medicine, it is common that susceptible individuals leave the workplace early due to symptoms, discomfort or acute illness. This may lead to selection bias with the remaining workers less sensitive or resistant to these noxious factors. Furthermore, we prospectively powered the study based on the measurements of primary end points made during previous studies.(Mills et al., 2005, Newby et al., 1998, Newby et al., 1999, Lucking et al., 2010) Although we are confident that we have not missed effects on endothelial function or ex vivo thrombosis, we acknowledge that we may have had insufficient power to detect modest changes in some of the secondary end points, and thus cannot exclude the possibility of false-negative findings confounding their assessment. Nevertheless, even allowing for these limitations, in a carefully designed and controlled study with a comprehensive assessment of cardiovascular function, we found no adverse effects of exposure to wood smoke.

4.6 CONCLUSION

Isolated wood smoke exposure at concentrations occurring in the vicinity of major wildland fires did not impair vascular vasomotor or fibrinolytic function, or increase thrombus formation in firefighters. The acute cardiovascular events associated with fire suppression may not be directly related to wood smoke exposure, rather they may be precipitated by other pollutants or mechanisms such as strenuous physical exertion and dehydration.

CHAPTER 5

EFFECT OF HEAT AND PHYSICAL EXERTION ON VASCULAR FUNCTION AND THROMBUS FORMATION IN HEALTHY FIREFIGHTERS

Extracts from this chapter have been published in:

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Chapter 5: EFFECT OF HEAT AND PHYSICAL EXERTION ON VASCULAR FUNCTION AND THROMBUS FORMATION IN HEALTHY FIREFIGHTERS

5.1 SUMMARY

Rates of myocardial infarction in firefighters are increased during fire suppression duties, and are likely to reflect a combination of factors including extreme physical exertion and heat exposure. We assessed the effects of simulated fire suppression on measures of cardiovascular health in healthy firefighters.

In an open-label randomized cross-over study, nineteen healthy firefighters (41±7 years, 16 male) performed a standardized training exercise in a fire simulation facility or light duties for 20 min. Following each exposure, *ex vivo* thrombus formation, fibrinolysis, platelet activation, and forearm blood flow in response to intra-arterial infusions of endothelial-dependent and -independent vasodilators were measured.

Following fire simulation training, core temperature increased (1.0±0.1 °C) and weight reduced (0.46±0.14 kg, P<0.001 for both). Compared to control, exposure to fire simulation increased thrombus formation under low-shear (73±14 %) and high-shear (66±14 %) conditions (P<0.001 for both), and increased platelet-monocyte binding (7±10 %, P=0.03). There was a dose-dependent increase in forearm blood flow with all vasodilators (P<0.001), that was attenuated by fire simulation in response to acetylcholine (P=0.01) and sodium nitroprusside (P=0.004). This was associated with a rise in fibrinolytic capacity, asymptomatic myocardial ischemia, and an increase in plasma cardiac troponin I concentrations (2.2±0.4 *versus* 4.2±1.0 ng/L, P=0.009).

Exposure to extreme heat and physical exertion during fire suppression activates platelets, increases thrombus formation, impairs vascular function and promotes myocardial ischemia and injury in healthy firefighters. Our findings provide pathogenic mechanisms to explain the association between fire suppression activity and acute myocardial infarction in firefighters.

5.2 INTRODUCTION

Cardiovascular events are the leading cause of death amongst firefighters and are responsible for approximately 45% of on-duty fatalities each year in the United States (Kales et al., 2003). These deaths disproportionately cluster around fire-suppression duties, despite this activity accounting for only 1-5 % of a firefighter's time (Kales et al., 2007). Death from coronary artery disease was 12- to 136-times more likely to occur during or shortly after fire suppression than non-emergency duties. The hostile conditions of fire suppression include high ambient temperatures, extreme physical exertion, noxious air pollutants and psychological stress. It is likely there are additive or synergistic effects of these potential triggers in susceptible firefighters that may culminate in acute cardiovascular events.

Fire training forms an important part of the recruitment process and training amongst operational firefighters. The cardiovascular response to fire suppression is difficult to study in a real-life setting due to the unpredictability of events and time pressures during an emergency situation. As a research tool, real fire training centers offer a unique opportunity to assess the physiological effects of fire-suppression in a controlled setting (Angerer et al., 2008a, Burgess et al., 2012, Fahs et al., 2011, Horn et al., 2013, Smith et al., 2014, Smith et al., 1997, Ljubicic et al., 2014, Del Sal et al., 2009, Smith et al., 2011, Smith et al., 2001, Smith et al., 1996, Smith and Petruzzello, 1998, Smith et al., 2005). Previous observations suggest that heat and physical

stress, in combination with hemoconcentration due to fluid loss, reduce cardiac output and induce a hypercoagulable state (Angerer et al., 2008, Smith et al., 2001). However, our understanding of the pathogenic mechanisms to explain the association between fire suppression and acute myocardial infarction is incomplete and therefore the optimal approach to reduce risk in firefighters is uncertain. Our aim was to undertake a comprehensive assessment of the effects of fire suppression on cardiovascular health in firefighters.

5.3 METHODS

5.3.1 STUDY PARTICIPANTS

Nineteen healthy non-smoking firefighters were enrolled into the study. The study was performed in accordance with the Declaration of Helsinki, with the approval of the local research ethics committee and the written informed consent of all volunteers. Subjects were recruited by sending study information sheets and letters to randomly selected firefighters from the Scottish Fire and Rescue Service. Exclusion criteria included cigarette smoking, known cardiovascular disease, arrhythmias, diabetes mellitus, hypertension, asthma, use of regular medication, renal or hepatic impairment, or an inter-current infective illness. Subjects reported no symptoms of respiratory tract infection within the 4-week period preceding the study.

5.3.2 STUDY DESIGN

Subjects attended on two occasions, at least one week apart, and participated in a standardized training exercise in a fire simulation facility (exposure) or undertook light duties (control) in an open-label, randomized crossover design. Firefighters attended following a period of 48 hrs off-duty to minimise the impact of confounding from other occupational exposures. Fire simulation exposure was performed at a separate dedicated training

facility prior to transport to the clinical research facility. During the control period, firefighters attended the clinical research facility and were permitted to undertake light activity similar to those performed during a shift without emergency duties (***Figure 5.1***).

Following each exposure, cardiovascular assessments were performed in a quiet, temperature-controlled room maintained at 22°C to 24°C with subjects lying supine. All subjects abstained from alcohol for 24 h and from food, tobacco, and caffeine-containing drinks for at least 4 h before each vascular study. Female subjects were assessed at the same time point of their menstrual cycle.

The primary endpoints were *ex vivo* thrombus formation, forearm blood flow, and net tissue plasminogen activator (t-PA) release. Secondary endpoints were platelet activation assessed by flow cytometry, differential cell count, plasma urea, creatinine, lactate, glucose and cardiac troponin I concentrations, ambulatory heart rate and blood pressure monitoring, and ischemic burden on 12-lead Holter monitoring.

Based on previous studies (Mills et al., 2005, Lucking et al., 2008, Lucking et al., 2011, Langrish et al., 2010), *ex vivo* thrombus formation was assessed 1-2 h after each exposure, and forearm blood flow and t-PA release were

assessed 2-4 h after each exposure. Blood samples were obtained at baseline, immediately, 4 h and 24 h post-exposure.

Subjects were fitted with a portable 12-lead electrocardiogram (Lifecard CF, Delmar Reynolds Medical Ltd, UK) and blood pressure monitor (Spacelabs 90217, Spacelabs, UK) at least 30 min prior to and for 24 h post exposure. On the evening prior to the fire simulation training, firefighters were asked to swallow an ingestible temperature monitor (CorTemp, HQInc, USA). In cases where the pill was no longer in their gastrointestinal tract when arriving at the study site, subjects swallowed a second pill approximately 30 min prior to study commencement. Core body temperature was measured continuously with externally worn temperature loggers (CorTemp, HQInc, USA) from 30 min prior to exposure, throughout the exposure and for at least 6 h thereafter. All subjects wore full personal protective equipment (PPE) and self-contained breathing apparatus (SCBA) for the fire simulation exposure.

Overall sweat loss was determined by the difference in body mass between beginning and end of the exposure, and was corrected for any fluid consumption. Firefighters voided prior to being weighed before the fire simulation exposure. Participants reported their ratings of perceived exertion (RPE), from 6–20 (*very light* to *very, very difficult*) on the Borg scale immediately post exposure (Borg, 1982).

5.3.3 EXPOSURES

Firefighters attended the Scottish International Fire Training Centre, Edinburgh for the fire simulation exposure as described previously in Chapter 2.

5.3.4 EX-VIVO THROMBUS STUDIES

Thrombus formation was measured using the Badimon chamber as described previously in Chapter 2.

5.3.5 FLOW CYTOMETRY

Samples were obtained at baseline, at 2 h immediately prior to the thrombosis study and at 24 h post exposure, and processed as described previously in Chapter 2.

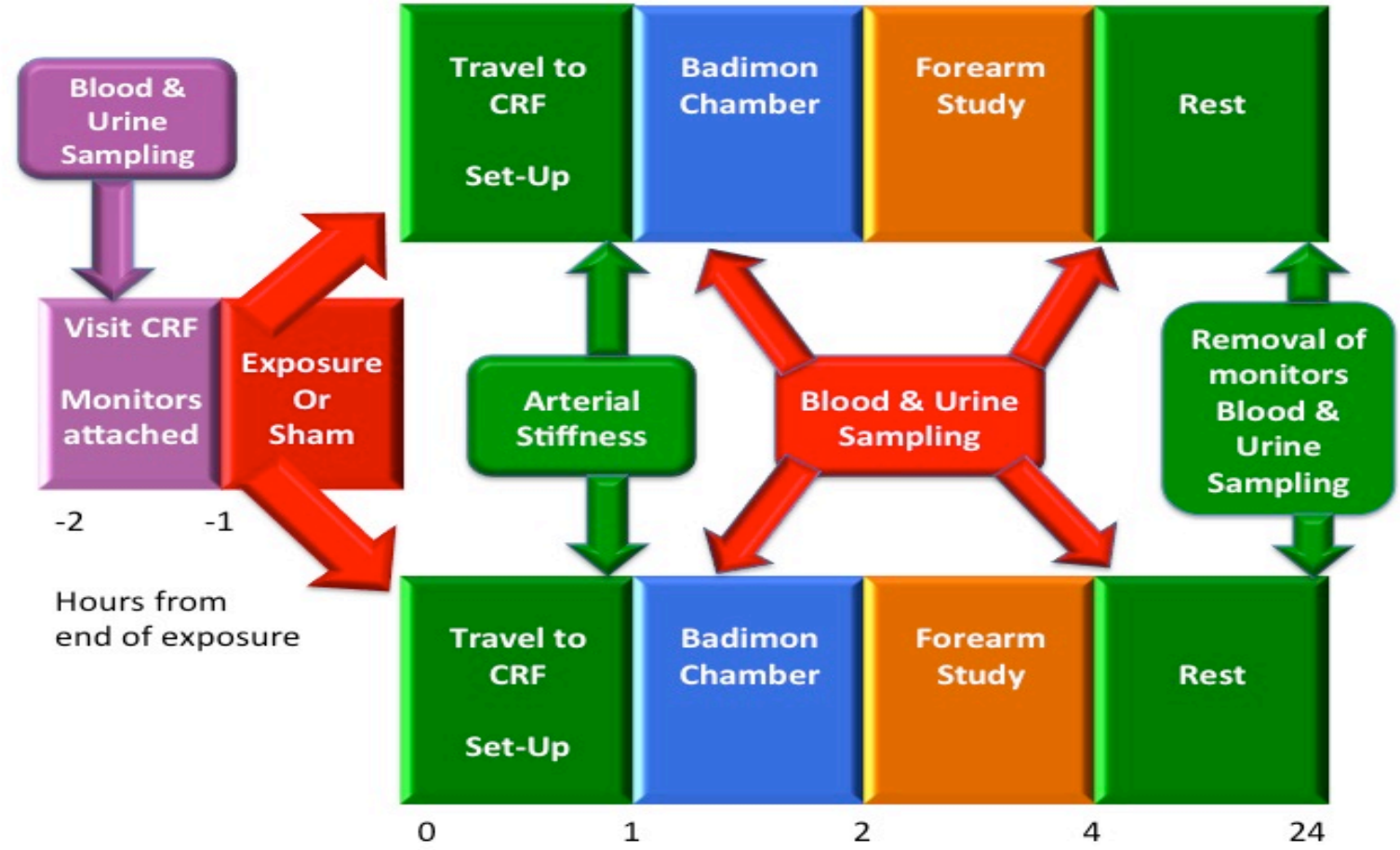


Figure 5-1 Study Design

5.3.6 VASOMOTOR AND FIBRINOLYTIC STUDIES

All subjects underwent venous occlusion plethysmography and plasma t-PA and PAI-1 antigen concentrations were determined as described previously in Chapter 2.

5.3.7 ASSAYS

Venous blood was analyzed for total cells, differential cell counts, and platelets by an auto-analyzer and for plasma urea and electrolytes, lactate, and glucose concentration in the regional laboratories at the Royal Infirmary of Edinburgh. Plasma cardiac troponin I concentrations were determined using a high-sensitivity assay (ARCHITECT$_{STAT}$, Abbott Diagnostics, Chicago, USA) as described previously in Chapter 2.

5.3.8 ECG ANALYSIS

Electrographic recordings were analysed with the use of the Medical Pathfinder Digital 700 Series Analysis System (Delmar Reynolds Medical Ltd, UK) as described previously in Chapter 2.

5.3.9 DATA ANALYSIS AND STATISTICS

Continuous variables are reported as mean±standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism, version 5.0 (Graph Pad Software, USA) by 2-way analysis of variance (ANOVA) with repeated measures and 2-tailed Student's paired *t*-test, or Wilcoxon signed-rank as appropriate. Cardiac troponin I concentrations were log transformed prior to analysis. Statistical significance was taken at two-sided $P<0.05$.

5.4 RESULTS

Nineteen healthy non-smoking firefighters (mean age 41±7 years, 16 males) were enrolled. Seventeen firefighters had both exposures with two firefighters unable to complete the study.

The average maximum temperature in the fire simulation facility was 406.2±13.5 °C. Mean core temperature was at 37.4±0.1 °C baseline and peaked at 38.4±0.1 °C. Body weight reduced by 0.46±0.14 kg. Heart rate and temperature rose rapidly and was associated with asymptomatic ST-segment depression during fire simulation exposure (ANOVA P<0.01 for all, ***Figure 5.2***). Perceived exertion was rated as 14 ± 0.2 on the Borg scale.

5.4.1 THROMBUS FORMATION AND PLATELET ACTIVATION

Compared to the control period, thrombus formation was increased following fire simulation exposure by 73% in the low-shear chamber (change in thrombus area 5,781 μm^2, 95% confidence interval [CI] 3,340-8221 μm^2; P<0.001) and by 66% in the high shear chamber (change in thrombus area 6,563 μm^2, 95% CI 3,481-9645 μm^2; P<0.001, ***Figure 5.3***). Platelet-monocyte aggregation differed at baseline between control and fire simulation exposure, but was increased following fire simulation (7%, 95% CI 0-13%; P=0.03) and was unchanged across the control period (-6%, 95% CI -15-1%; P=0.09, ***Figure 5.3***). Platelet surface expression of P-selectin and

CD40 ligand were similar following control and fire simulation exposure

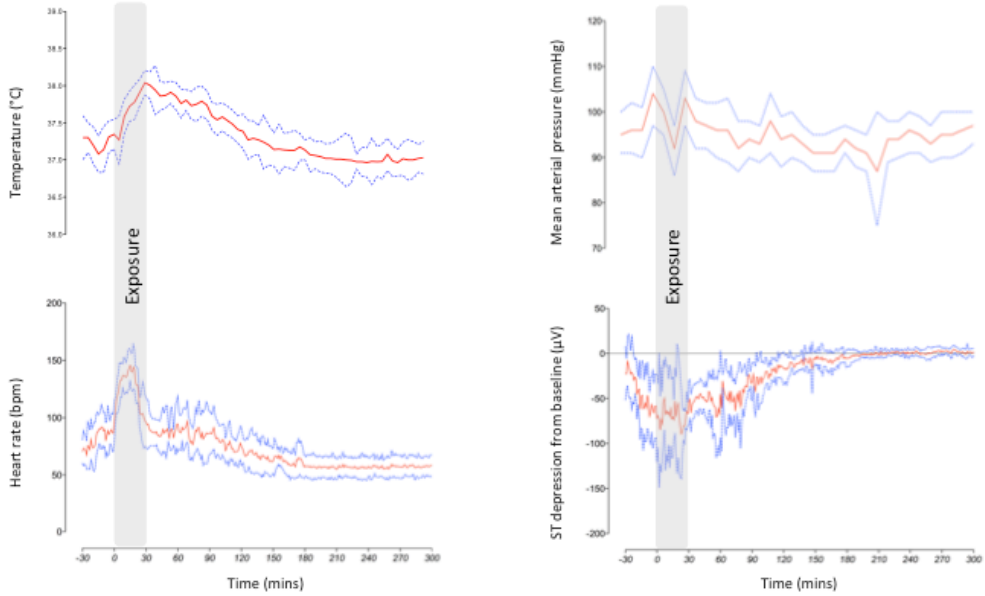
(P>0.05 for both).

Table 5-1. Secondary end-points before and after control and fire simulation exposures

	Control (n=17)				Fire simulation (n=17)				P value[1]
	Pre exposure	*Post exposure*	*4 hrs*	*24 hrs*	*Pre exposure*	*Post exposure*	*4 hrs*	*24 hrs*	
Haemoglobin g/L	139 ± 3	138 ± 3	142 ± 3	142 ± 3	144 ± 3*	149 ± 3*	137 ± 2†	141 ± 3	0.002
Haematocrit	0.41 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.42 ± 0.01†	0.43±0.01*	0.40 ± 0.01†	0.41 ± 0.01	0.02
Leucocytes x 10^9 cells/L	5.5 ± 0.3	5.9 ± 0.2	6.1 ± 0.2	5.7 ± 0.2	6.1 ± 0.4	7.4 ± 0.6†	7.5 ± 0.4‡	5.7 ± 0.3	<0.001
Platelets x 10^9 cells/L	241 ± 11	234 ± 10	226 ± 12	253 ± 11	262 ± 10	305 ± 15*	231 ± 8	259 ± 11	<0.001
Sodium mmol/L	139 ± 0.5	139 ± 0.5	139 ± 0.5	140 ± 0.6	139 ± 0.4	139 ± 0.6	139 ± 0.4	139 ± 0.5	0.02
Potassium mmol/L	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	0.1
Bicarbonate mmol/L	23 ± 1.5	25 ± 0.4	24 ± 0.5	23 ± 0.6	25 ± 0.4	20 ± 0.7*	23 ± 0.3	23 ± 0.4	0.03
Urea mmol/L	5.0 ± 0.3	4.9 ± 0.3	4.5 ± 0.2	5.1 ± 0.3	5.0 ± 0.3	5.1 ± 0.3	4.8 ± 0.3	5.2 ± 0.2	0.16
Creatinine mmol/L	74 ± 3	74 ± 3	70 ± 2	78 ± 3	76 ± 2	84 ± 3*	71 ± 2	77 ± 3	<0.001
Lactate mmol/L	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	-	1.4 ± 0.2	3.7 ± 0.5*	1.0 ± 0.0	-	<0.001
Glucose mmol/L	4.8 ± 0.1	4.7 ± 0.1	-	-	4.5 ± 0.2	6.0 ± 0.2*	-	-	0.007
t-PA ng/mL	8.7 ± 1.2	8.4 ± 1.0	-	-	7.6 ± 1.1	7.1 ± 1.4	-	-	0.04
PAI 1 ng/mL	-	3.0 ± 0.6	-	-	-	2.7 ± 0.4	-	-	0.08
t-PA/PAI 1 ratio	-	2.9 ± 0.5	-	-	-	3.8 ± 0.7	-	-	0.16
Cardiac troponin I ng/L	2.6 ± 0.4	2.2 ± 0.4	-	2.0 ± 0.4	1.8 ± 0.3	4.6 ± 1.0*	-	3.6 ± 0.8	0.009

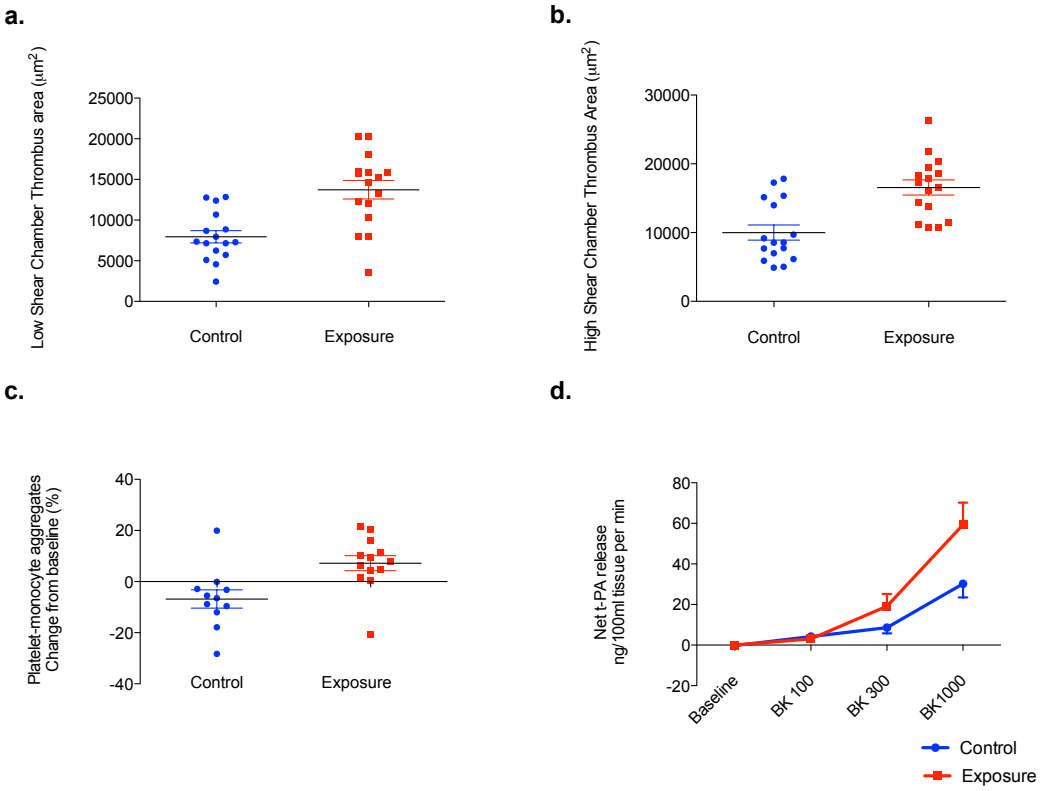
*Values are reported as mean ± SEM; 2-way ANOVA with repeated measures comparing control and fire simulation exposures. *P<0.001 following Bonferroni correction comparing control and fire simulation exposures at a given time point. †P<0.01 following Bonferroni correction comparing control and fire simulation exposures at a given time point. ‡P<0.05 following Bonferroni correction comparing control and fire simulation exposures at a given time point.*

Figure 5-2. Core temperature and hemodynamic changes during fire simulation exposure



Core temperature and heart rate rapidly increased during the fire simulation accompanied by asymptomatic ST-segment depression. Core temperature remained increased for 120 min and ST-segments returned to baseline gradually during the same time period. Mean arterial pressure was unchanged throughout. Values are mean ± 95% CI.

Figure 5-3. Thrombus formation and platelet activation following fire simulation exposure



Thrombus formation ex vivo was increased in response to fire simulation in under both low shear and high shear conditions compared to control (student's t-test, P<0.001 for both, panel A and B respectively). Platelet monocyte aggregates were increased following fire simulation exposure compared to control (Student's t-test, P=0.004, n=12, panel C). Where venepuncture was challenging and resulted in significant ex-vivo platelet activation (>80%) subjects were excluded from analysis. Where this occurred data was excluded for both visits to avoid any bias. There was a dose-dependent increase in tissue-plasminogen activator in response to bradykinin in both exposures (2-way ANOVA with repeated measures, P<0.001), that was augmented following fire simulation compared to control (ANOVA, P=0.006, panel D).

5.4.2 VASCULAR VASOMOTOR AND FIBRINOLYTIC FUNCTION

Following fire simulation exposure both systolic and diastolic blood pressures were lower immediately prior to the vascular studies when compared to the control period (systolic blood pressure 125±2 *versus* 134±3 mmHg, diastolic blood pressure 75±2 *versus* 82±2 mmHg; P<0.01 for both). Basal forearm blood flow was higher at baseline following fire simulation exposure compared with the control period (2.3±0.2 *versus* 1.7±0.1 mL/100 mL/min; P=0.01). Following administration of acetylcholine, bradykinin, sodium nitroprusside and verapamil, there were dose-dependent increases in forearm blood flow after both fire simulation and control periods (P<0.001, ***Figure 5.4***). Vasodilatation expressed as a ratio of forearm blood flow in the infused and control arms was attenuated in response to acetylcholine (P=0.01) and sodium nitroprusside (P=0.004) compared to control, but was unaffected by bradykinin or verapamil infusions (P>0.05 for both).

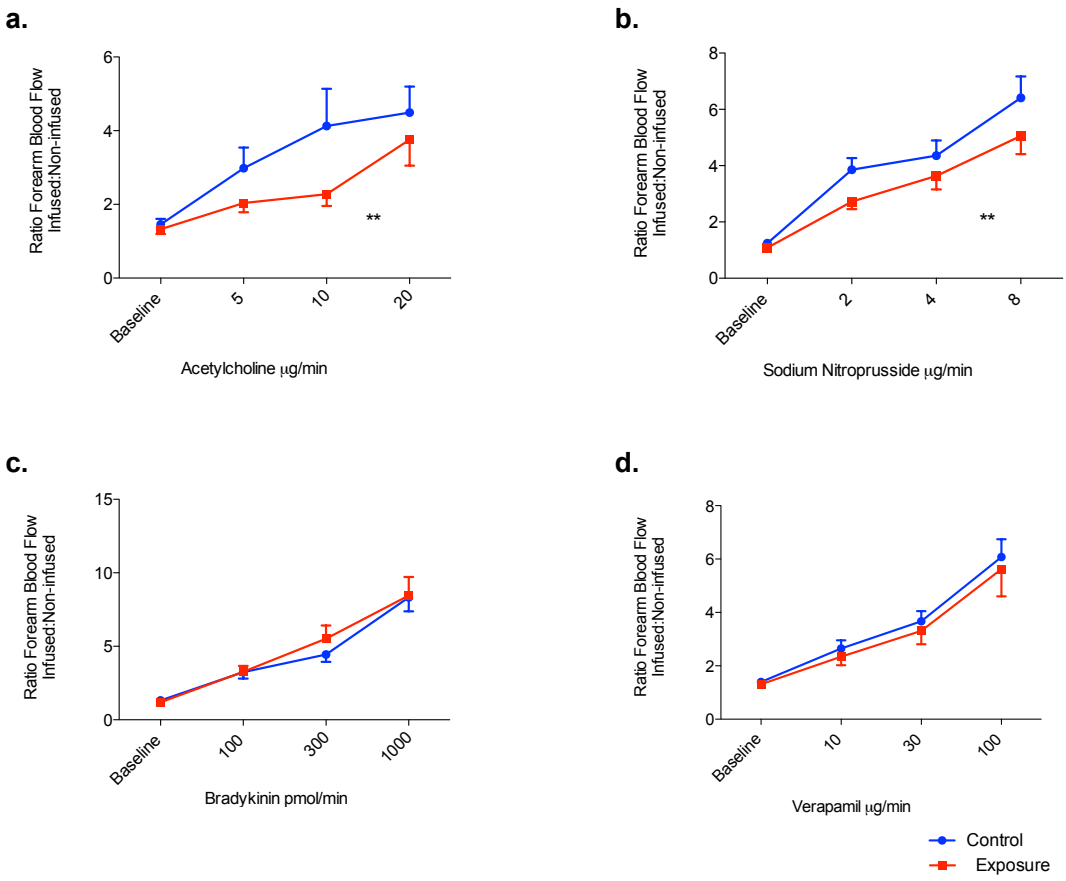
Bradykinin caused a dose-dependent increase in plasma t-PA antigen concentrations (P<0.001). Following fire simulation exposure, there was a doubling of the net release of t-PA antigen compared to control (area under the curve [AUC] 51.9 *versus* 27.9 ng/100 mL/min; P=0.006, ***Figure 5.3***).

5.4.3 SECONDARY ENDPOINTS

There were increases in haemoglobin, haematocrit, platelets and total leucocytes immediately following fire simulation compared to control (haemoglobin 149±3 *versus* 138±3 g/L, haematocrit 0.43±0.01 *versus* 0.40±0.01, platelets 305±15 *versus* 234±10 x 10^9 cells/L, total leucocytes 7.4±0.6 *versus* 5.9±0.2 x 10^9 cells/L; P<0.05 for all, ***Table 1***). Total leucocytes remained elevated at 6 h post exposure when compared with the control period (7.5±0.4 *versus* 6.1±0.2 x 10^9 cells/L; P<0.05). Serum lactate was markedly increased immediately following fire simulation exposure reflected by a corresponding fall in bicarbonate concentrations (lactate 3.7±0.5 *versus* 1.0±0.1 mmol/L, bicarbonate 20±0.7 *versus* 25±0.4 mmol/L; P<0.05 for both).

High-sensitivity cardiac troponin I concentration increased 1 h following fire simulation compared to control (4.2±1.0 *versus* 2.2±0.4 ng/L, P=0.009). There was a significant increase in the number of ischemic events (0.5 mm), maximum ST-segment depression and cumulative ischaemic burden during fire simulation exposure as compared to the control period (P<0.05 for all), but these parameters did not differ across the subsequent 23-h period (***Table 2***).

Figure 5-4. Vascular vasomotor function following fire simulation exposure



There was a dose dependent increase in forearm blood flow with each vasodilator (2-way ANOVA with repeated measures P<0.001 for all). Vasodilatation expressed as a ratio of the forearm blood flow between the infused and non-infused arm, was attenuated in response to acetylcholine and sodium nitroprusside (P=0.01 and P=0.004, panel A and B respectively) following fire simulation compared to control. There was no difference in forearm blood flow in response to bradykinin or verapamil (P>0.05 for both, panel C and D respectively) between the two exposures.

Table 5-2 Continuous 12-lead electrographic analysis

		During & 1 h post exposure			23 h post exposure		
		Control *(n=17)*	**Fire simulation** *(n=17)*	**P value**	**Control** *(n=17)*	**Fire simulation** *(n=17)*	**P value**
Max HR, bpm		75 ± 4	162 ± 4	<0.0001	121 ± 7	123 ± 6	0.34
0.5mm events	**Lead II**	1	18	0.005	85	78	0.92
	Lead V2	0	9	0.03	78	75	1.0
	Lead V5	1	12	0.006	69	59	0.62
	Total	2	39	<0.0001	232	212	0.75
Max ST depression μV	**Lead II**	-26 ± 22	-361 ± 26	<0.0001	-263 ± 32	-229 ± 37	0.41
	Lead V2	-35 ± 20	-290 ± 54	0.003	-235 ± 91	-349 ± 63	0.43
	Lead V5	-12 ± 20	-255 ± 71	0.01	-193 ± 45	-206 ± 94	0.84
	Total	-23 ± 12	-304 ± 32	<0.0001	-231 ± 32	-256 ± 39	0.97
Ischaemic burden mV/s	**Lead II**	-12 ± 10	-207 ± 22	0.002	-1126 ± 297	-888 ± 168	0.38
	Lead V2	-22 ± 14	-258 ± 128	0.03	-1320 ± 569	-1424 ± 206	0.84
	Lead V5	-15 ± 10	-139 ± 36	0.02	-536 ± 315	-583 ± 101	0.67
	Total	-16 ± 6	-192 ± 33	<0.0001	-1005 ± 229	-958 ± 116	0.92

Values are reported as mean ± SEM; Student's paired t-test and Wilcoxon signed-rank test comparing control and fire simulation exposures.

5.5 DISCUSSION

Following a short exposure to fire simulation training, we demonstrate striking changes in physiological measures of cardiovascular function. This is the first study to demonstrate that participation in fire simulation results in increased thrombus formation along with enhanced platelet activation and impairment of vasomotor endothelial function: all key mechanisms in the pathogenesis of acute myocardial infarction. Intriguingly, fire simulation was associated with evidence of minor myocardial injury and asymptomatic myocardial ischemia. Taken together, this comprehensive cardiovascular assessment has detected plausible mechanistic links between participation in fire suppression duties and acute myocardial infarction.

Participation in fire simulation training places an inordinate strain on the cardiovascular system. By undertaking a comprehensive physiological assessment that included continuous core body temperature and hemodynamic monitoring, we are able to demonstrate the dramatic effect that heat, and physical exertion has on a range of physiological and cardiovascular health measures. We reveal a substantial loss of body weight secondary to dehydration in association with a marked temperature rise of 1 °C, lactic acidosis and leucocytosis. Unsurprisingly, this was associated with an acute increase in heart rate and relative hypotension over the following 3-4 hours as the subjects returned to normothermia.

For the first time, we demonstrate an increase in *ex vivo* thrombus formation in response to fire simulation exposure which is perhaps not surprising given the associated hemoconcentration. The Badimon chamber is a well-validated *ex vivo* model of thrombosis permitting measurement of thrombus formation in native whole blood in conditions which simulate coronary arteries following plaque disruption (Lucking et al., 2008, Lucking et al., 2010, Badimon et al., 1987). The degree of thrombus formation demonstrated in healthy firefighters following a short fire simulation exposure is somewhat extraordinary. Our group have previously reported an increase in *ex vivo* thrombus formation following diesel exhaust exposure (Lucking et al., 2008) but the degree of thrombus formation in this current study was over three-fold greater. This is likely to be explained by systemic inflammation together with presumed sympathetic activation, the combination of which stimulates platelets and activates coagulation pathways. Moreover, the increased thrombogenicity due to dehydration further compounds this effect resulting in a substantial increase in thrombus formation. Others have previously demonstrated a 24% increase in platelet count and an increase in platelet aggregability (Smith et al., 2011). Alongside demonstrating a 31 % increase in platelet count, we employ a more robust assessment of platelet activation by flow cytometry and have demonstrated an increase in platelet-monocyte aggregates following fire simulation suggesting that increased thrombus formation is at least in part mediated by platelet activation.

We observed an increase in endothelial tissue plasminogen activator release from the vascular endothelium in response to intra-arterial bradykinin infusion following fire simulation exposure and this is likely to be compensatory in response to the marked prothrombotic state. Previous studies have shown that plasma t-PA antigen concentrations increase in parallel with increased coagulation in the early phase following both sub-maximal exercise (Hegde et al., 2001, Lin et al., 1999) and a similar fire simulation exposure (Smith et al., 2014). However, this fibrinolytic response diminishes within 2 h with a persistent imbalance of thrombosis and fibrinolysis in favour of a prothrombotic state. This perhaps explains the ongoing susceptibility to cardiac events in firefighters beyond the immediate post-exposure period. Furthermore, it has been previously established that creating a pro-inflammatory state in the vascular endothelium results in a sustained and substantial increase in endothelial tissue plasminogen activator whilst simultaneously impairing endothelial vasomotor function (Chia et al., 2003). A limitation of our study is that we did not assess t-PA release at a later time point, when any systemic inflammatory response to fire simulation is likely to be more marked.

Previous work by Tei and colleagues has demonstrated that exposure to heat alone in the form of a hot water bath or sauna, increased core body temperature by 1.2°C and reduced systemic vascular resistance during and for up to 30 min following exposure in patients with heart failure (Tei et al.,

1995). The authors conclude that regular heat exposure could have a beneficial effect on cardiovascular physiology in these patients. Although we demonstrate a similar increase in core temperature following fire simulation, the additional physical and psychological effects of fire suppression are distinct. Furthermore, we evaluated vascular function 2 h following exposure when core body temperature had nearly returned to baseline. In a similar setting to our study, Fahs and colleagues have previously revealed an increase in arterial stiffness together with increases in forearm blood flow and reactive hyperaemia following fire simulation as measured non-invasively by venous occlusion plethysmography (Fahs et al., 2011). Limited conclusions were drawn from this study given the apparently opposing vascular effects demonstrated. In contrast, we have employed venous occlusion plethysmography with intra-arterial infusion of vasodilators, widely regarded as the ‘gold-standard’ assessment of endothelial vasomotor function. Although forearm blood flow following fire simulation exposure was elevated due to systemic vasodilatation, we demonstrate a detrimental effect on endothelium-dependent and -independent vasodilatation following exposure to fire simulation by reporting change in blood flow as a ratio of the infused to non-infused arms to account for systemic vasodilatation.

The endothelium is a major target for inflammation and consequent oxidative stress with impairment of endothelial vasomotor function being associated with an increased risk of acute cardiovascular events, including

cardiovascular death (Schächinger et al., 2000, Al Suwaidi et al., 2000). It is conceivable that the pro-inflammatory state created by exposure to fire simulation accounts for the attenuated response to acetylcholine and sodium nitroprusside whose vasodilatory actions are mediated by nitric oxide. We postulate that oxygen free radicals scavenge nitric oxide thus reducing its bioavailability. By contrast, there was no impairment of vasomotor function with bradykinin or verapamil following either exposure. This would suggest that impaired vasodilatation to acetylcholine and sodium nitroprusside is not simply a manifestation of altered basal tone or systemic vasodilatation. Bradykinin causes vasodilatation primarily through the release of endothelium-derived hyperpolarising factor and prostaglandins, and therefore the vasomotor response to bradykinin infusion may be less susceptible to the acute effects of oxidative stress. Alternatively, active vasodilatation during whole body heat stress may be mediated by the cyclooxygenase pathway (McCord et al., 2006) which could also explain the lack of attenuation in forearm vasomotor response to bradykinin following fire simulation exposure. It is likely that vasodilatation is mediated by upregulated prostanoids in this setting and counteracts the impairment of nitric oxide mediated dilatation resulting in a neutral response to bradykinin administration.

We have demonstrated small increases in plasma high-sensitivity troponin I concentrations following fire suppression exposure. Previous studies have reported a link between endurance exercise and cardiac troponin release

(Regwan et al., 2010, Fortescue et al., 2007, Sedaghat-Hamedani et al., 2015). The magnitude of increase in troponin post-exercise is related to exercise intensity and cardiovascular physiology. In a recent meta-analysis, there was a pooled increase in cardiac troponin I from baseline of 40 ng/L (95% CI 21.4-58 ng/L) and in cardiac troponin T of 26 ng/L (95% CI 5.2-46) following prolonged endurance exercise with a mean exercise duration of 229 min (Sedaghat-Hamedani et al., 2015). We used a high-sensitivity cardiac troponin I assay with excellent precision at very low concentrations in this study (Shah et al., 2015, Chin et al., 2014) and were able to observe a small increase in cardiac troponin I concentrations in all subjects after only 20 min of fire suppression training. In addition, we demonstrate ST-segment depression on ambulatory monitoring and periods of asymptomatic myocardial ischemia during and immediately following fire simulation exposure. The firefighters in this study were healthy with no risk factors for, and no known underlying coronary artery disease. While cardiac troponin I concentrations remained within the normal reference range and the degree of myocardial ischemia was relatively small, it is plausible that these changes represent direct cardiac injury and the cardiotoxic effect of cytokines such as tumour necrosis factor, heat shock protein, or oxygen free radicals. Alternatively this may represent an oxygen supply-demand mismatch causing myocardial injury at the extremes of physical exertion (Lim et al., 2006). Further studies would be required comparing the effects of fire suppression with the effects of an equivalent period of physical exercise in

the absence of fire suppression to determine the mechanism of myocardial injury.

The measured maximal heart rates in this study were similar to other fire simulation studies (Del Sal et al., 2009, Smith et al., 2001, Manning and Griggs, 1983, Sothmann et al., 1992, Angerer et al., 2008). Despite the apparent strenuous exertion, the fire simulation exposure was graded by subjects as *strenuous, yet not very hard* on the Borg Scale. Ratings of perceived exertion are commonly used in simulated real fire exercises. However, there is generally poor correlation between perceived exertion and heart rate with most subjects grading exercises as less strenuous than their heart rates would otherwise suggest. This raises an important safety issue and questions if firefighters are aware they are working at the limits of their physiological capabilities.

Fire simulation exposure undoubtedly is not accurately representative of real-life fire suppression which is the main limitation of this study. In real-life fire suppression, the physiological stresses demonstrated in this fire simulation will undoubtedly be compounded by uncontrolled and higher ambient temperatures, multiple entries into the same fire and the potential psychological stress of attending an unknown and dangerous situation where one's life and the lives of others are at risk. All firefighters involved in this study were familiar with the fire simulation centre and the exercise

undertaken owing to previous attendances for annual training. If we can extrapolate the findings of this study to a real-life fire suppression scenario, we would surmise that firefighters would have higher core temperatures given a higher ambient temperature which are unable to return to baseline given multiple entries to the same fire within a short time frame when there is often inadequate time for active cooling or rehydration prior to re-entry. Further studies are required outwith a fire training facility to assess the effects of real-life fire suppression which will encompass the additional triggers of psychological stress and air pollution, not assessed here, but undertaking such studies will undoubtedly prove logistically challenging. Additionally, although we have been able to demonstrate that the combination of extreme heat and physical exertion is detrimental to many measures of cardiovascular function we did not undertake a comparison of fire simulation exposure to an exposure consisting of either heat or exercise alone to assess the effect of each individual component on cardiovascular function. However, in this study we wished to simulate the effects of a real life fire suppression activity as closely as possible and in reality firefighters are never exposed to heat without physical exertion and neither are avoidable for them. Further experimental studies would be required to be undertaken to explore each of these components separately.

Our study has important implications for firefighters participating in fire simulation training. If the increased thrombogenicity and impaired vascular

function observed in our study is secondary to an increase in core body temperature and dehydration then limiting the duration of exposure, active cooling and effective rehydration would be simple and inexpensive ways to mitigate the risk posed by fire simulation training.

5.6 CONCLUSION

In conclusion, exposure to extreme heat and physical exertion during simulated fire suppression increases thrombogenicity, impairs vascular function and causes myocardial injury in healthy firefighters. Our findings suggest the pathogenic mechanisms to explain the association between fire suppression activity and acute myocardial infarction in susceptible firefighters

CHAPTER 6

EFFECT OF FIRE SUPPRESSION AND EMERGENCY DUTIES ON VASCULAR FUNCTION IN FIREFIGHTERS

Chapter 6: EFFECT OF FIRE SUPPRESSION AND EMERGENCY DUTIES ON VASCULAR FUNCTION IN FIREFIGHTERS

6.1 SUMMARY

Certain firefighter duties are associated with an increased risk of cardiovascular events. Fire suppression carries the greatest risk of cardiovascular events in on-duty firefighters with non-fire related alarm response and even physical activity related to increased risk of cardiovascular events compared with non-emergency duties. The mechanisms underlying increased risk are likely to be defined by the exposure to the constellation of occupational risk factors that these duties entail. By comparing cardiovascular health in firefighters following three distinct periods of duty we aim to further delineate the cardiovascular risk posed by occupational risk factors.

In a single-blind randomised cross-over study, 30 firefighters were recruited to attend following exposure to three distinct periods of duty: fire-suppression, alarm response without fire suppression and non-emergency activity. Following each exposure, *ex vivo* thrombus formation, and forearm

blood flow in response to intra-arterial infusions of endothelial-dependent and -independent vasodilators were measured. Sixteen firefighters completed at least one study visit, with 9 completing two study visits and a single firefighter completing all three visits.

Thrombus formation was similar following non-emergency activity and alarm response visits in both the low shear chamber (thrombus area 38,561 μm^2 *versus* thrombus area 31,620 μm^2 P = 0.40) and high shear chamber (thrombus area 32,801 μm^2 *versus* thrombus area 31,451 μm^2; P = 0.57). There was a dose-dependent increase in forearm blood flow with each vasodilator (P<0.01 for all). However, there were no differences in blood flow responses to acetylcholine (P=0.11), bradykinin (P=0.54), sodium nitroprusside (P=0.96) or verapamil (P=0.74) following non-emergency activity and alarm response visits.

To conclude, in this study comparing three distinct and representative periods of firefighter duty we have been unable to complete enough studies to adequately power an analysis and draw any firm conclusions about the exposures said duties entail. Further work is required in a real-world setting to more clearly define the occupational risk factors underlying the increased risk of cardiovascular events associated with specific firefighter duties.

6.2 INTRODUCTION

In the largest analysis of the cause of on-duty death amongst firefighters, 1,144 deaths over a 10 year period from 17 large metropolitan fire departments in the United States were examined (Kales et al., 2007). Deaths were classified according to the duty performed during the onset of symptoms or immediately prior to any sudden death. This analysis found that 32 % of cardiovascular deaths occurred during fire suppression, despite the fact that this activity only accounted for 1-5 % of the average firefighter's professional time per year. Death from coronary artery disease was 12- to 136-times more likely to occur during fire suppression as during non-emergency duties. Moreover, there was an increase, although to a lesser extent, in the risk of death associated with other specific emergency duties including alarm response (odds ratio 2.8 to 14.1), alarm return (odds ratio 2.2 to 10.5) and physical exertion (odds ratio 2.9 to 6.6). In comparison to other professions with similar responsibilities including emergency call-outs, physical exertion, and dangerous duties, firefighters have an abnormally high incidence of on-duty cardiovascular death. Perhaps what is unique to the fire service is the extreme working environment.

The exact biological mechanisms responsible for the increased risk of on-duty cardiovascular death remains unclear. Previous studies have been limited by the effect of potential confounding factors, and a lack of

mechanistic data. In this body of research we have been able to demonstrate that healthy firefighters do not have any vascular vasomotor or fibrinolytic function or increased thrombus formation off-duty and that wood smoke, as a form of air pollution frequently encountered by firefighters worldwide, also does not appear to cause impaired vascular vasomotor or fibrinolytic function, or increased thrombus formation in healthy firefighters (Hunter et al., 2014). We have however demonstrated the detrimental vascular and prothrombotic effects of exposure to extreme heat and physical exertion in a fire simulation exposure (Hunter et al., 2017) which does contribute somewhat to the understanding of the mechanisms involved in the evolution of a myocardial infarction following fire suppression. However, there is an increased, although lesser risk of death associated with other emergency duties. Ultimately, only an assessment of the effect of real-life firefighting duties will allow us to truly decipher the exact biological mechanisms at play and the occupational risk factors that increase the risks of cardiovascular death

By undertaking a comprehensive assessment of vascular function amongst healthy firefighters following real-life exposures to fire suppression and non-fire emergency duties and comparing to non-emergency duties, we attempt to further define the mechanisms underlying the increased risk associated with specific firefighting duties as described in the seminal epidemiological analysis by Kales et al (Kales et al., 2007). In doing so we hope to further

identify methods to protect on duty firefighters, and ultimately prevent premature cardiac deaths.

6.3 METHODS

6.3.1 STUDY PARTICIPANTS

Thirty healthy non-smoking firefighters were to be enrolled into the study. Due to the nature of the study and reliance on factors out with our control, such as the prevalence of exposure conditions, we recruited and screened thirty firefighters to ensure that at least twenty firefighters were able complete all three visits. All firefighters were invited for a screening visit where they had a clinical examination including 12-lead electrocardiogram and blood pressure measurement. Baseline blood tests were obtained for cell count, electrolytes, random glucose and lipid profile. The study was performed in accordance with the Declaration of Helsinki, with the approval of the local research ethics committee and the written informed consent of all volunteers. Exclusion criteria included cigarette smoking, known cardiovascular disease, arrhythmias, diabetes mellitus, hypertension, asthma, use of regular medication, renal or hepatic impairment, or an inter-current infective illness. Subjects reported no symptoms of respiratory tract infection within the 4-week period preceding the study.

6.3.2 STUDY DESIGN

The subjects were instructed to attend on three further occasions following three distinct periods of duty: fire-suppression, emergency or alarm response without fire suppression and following non-emergency activity i.e. sedentary shift. We randomised the order in which subjects attended (fire-suppression, alarm response, non-emergency duty) to ensure that any seasonal variation in the primary and secondary end-points did not influence the outcome. We could only use the Clinical Research Facility during working hours therefore the exposures studied occurred during the subjects' night-shifts.

Subjects attended immediately following relief from their shift and remained indoors in a controlled environment while awaiting vascular studies. Cardiovascular assessments were performed in a quiet, temperature-controlled room maintained at 22°C to 24°C with subjects lying supine. All subjects abstained from alcohol for 24 h and from food, tobacco, and caffeine-containing drinks for at least 4 h before each vascular study. Female subjects were assessed at the same time point of their menstrual cycle.

The primary endpoints were *ex vivo* thrombus formation and forearm blood flow. Based on previous studies (Mills et al., 2005, Lucking et al., 2008, Lucking et al., 2011, Langrish et al., 2010), *ex vivo* thrombus formation was assessed 1-2 h from the beginning of the study, and forearm blood flow was

assessed 2-4 h from the beginning of the study. Blood samples were obtained at baseline only (***Figure 6.1***).

Venous blood was analyzed for total cells, differential cell counts, and platelets by an auto-analyzer and for plasma urea and electrolytes, lipid profile, and glucose concentration in the regional laboratories at the Royal Infirmary of Edinburgh.

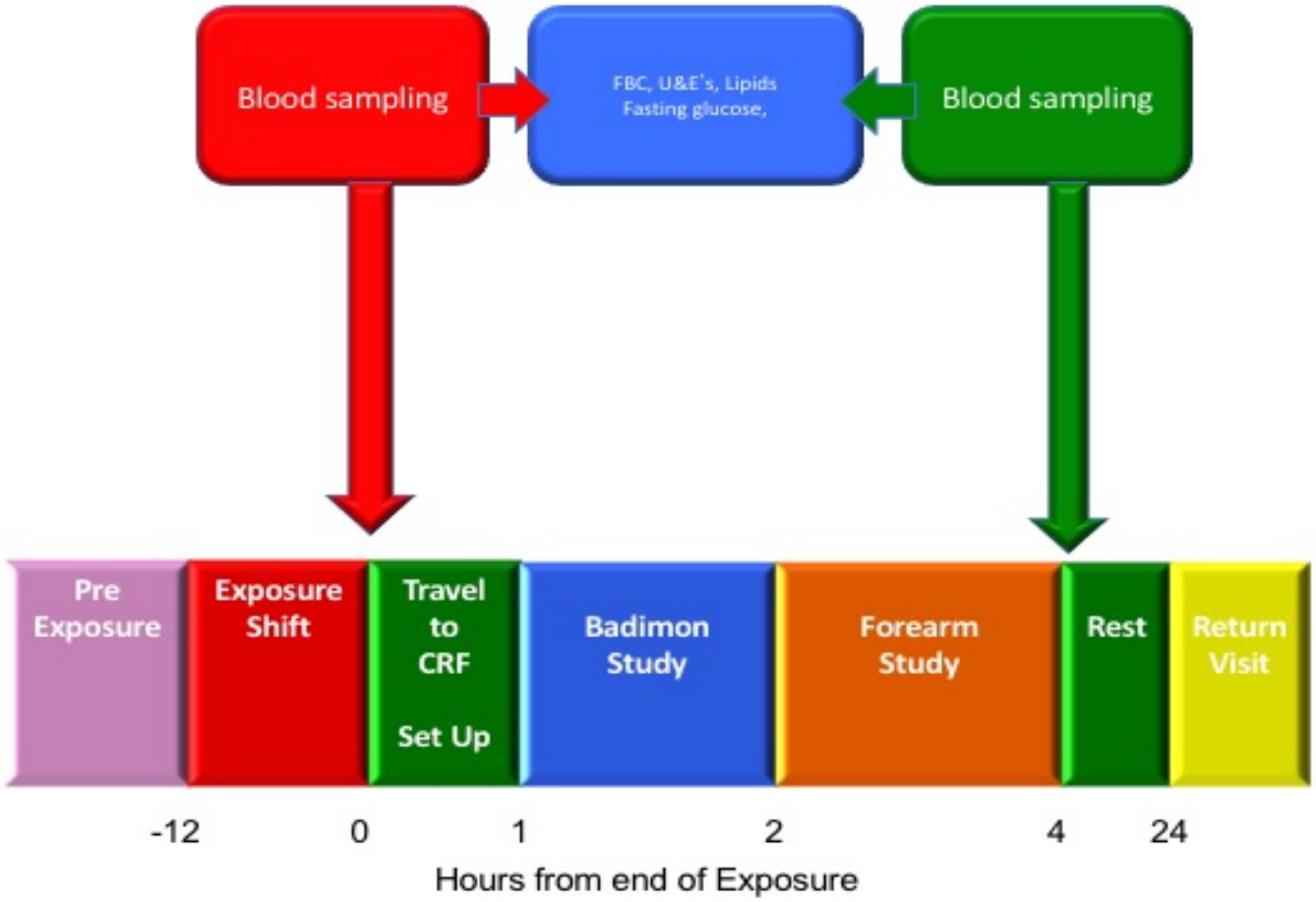
6.3.3 EX-VIVO THROMBUS STUDIES

Thrombus formation was measured using the Badimon chamber as described previously in Chapter 2.

6.3.4 VASOMOTOR STUDIES

All subjects underwent venous occlusion plethysmography as described previously in Chapter 2.

Figure 6-1 Study Design



6.3.5 DATA ANALYSIS AND STATISTICS

Continuous variables are reported as mean±standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism, version 5.0 (Graph Pad Software, USA) by 2-way analysis of variance (ANOVA) with repeated measures and 2-tailed Student's paired *t*-test, or Wilcoxon signed-rank as appropriate. Statistical significance was taken at two-sided $P<0.05$.

6.4 RESULTS

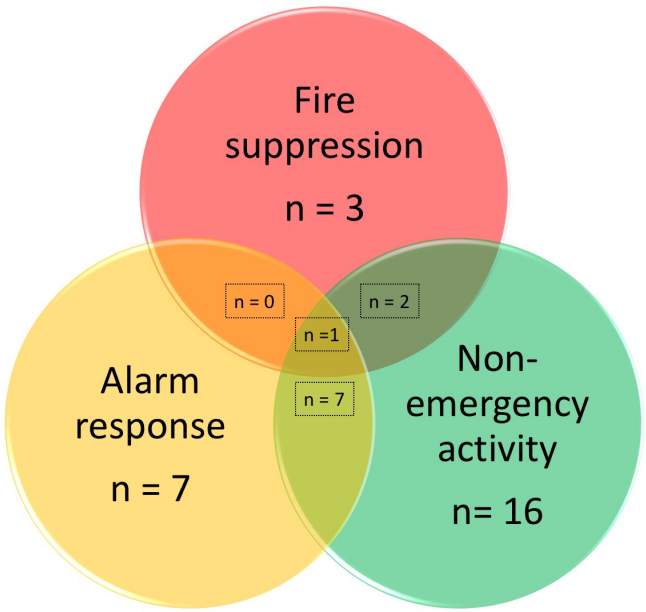
Thirty firefighters were recruited underwent screening for the study. Four firefighters were excluded from the study, three were discovered to have marked hypercholesterolaemia requiring treatment and the other wanted to continue to donate blood. A further firefighter decided that they could no longer commit to the study prior to any study visits. Of the remaining twenty-five firefighters, nine did not complete any study visits. The sixteen firefighters who completed at least one study visit between February 2012 and July 2014 are outlined in ***Figure 6.2,*** one of whom withdrew following his first and only study visit. The demographics of the firefighters all firefighters who were recruited to the study are described in ***Table 6.1***.

For the purposes of this chapter, I have elected to present a matched analysis of the alarm response and the non-emergency activity studies (n=7). I have displayed the fire suppression data (n=3) within ***Figures 6.3*** and ***6.4*** alongside the other data, but the sample size was not adequate to undertake a formal analysis.

Table 6-1 Demographics of Study Participants

Age, years	38 ± 1.7
Resting heart rate, bpm	61 ± 2
Systolic blood pressure, mmHg	135 ± 2
Diastolic blood pressure, mmHg	83 ± 2
BMI kg/m^2	26 ± 1
Fasting glucose, mmol/l	4.6 ± 0.1
Total cholesterol, mmol/l	4.8 ± 0.2
Cholesterol:HDLc ratio	3.8 ± 0.2

Figure 6-2 Study Visits



The number of study visits completed by type of exposure. The numbers in the middle describe how many subjects completed more than one study visit and what study visits they completed as indicated by the numbers in overlapping circles. There was one subject who completed all 3 study visits.

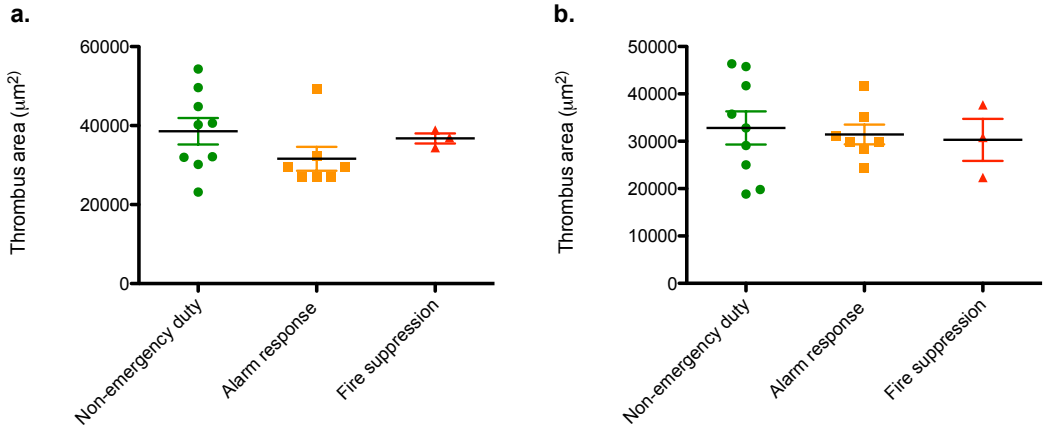
6.4.1 THROMBUS FORMATION

Thrombus formation was similar following non-emergency activity and alarm response visits in both the low shear chamber (thrombus area 38,561 μm^2, 95 % confidence interval [CI] 30,823 – 46,298 μm^2 *versus* thrombus area 31,620 μm^2, 95 % confidence interval [CI] 24,214 – 39,027 μm^2; P = 0.40) and high shear chamber (thrombus area 32,801 μm^2, 95% CI 24,751 – 40,852 μm^2 *versus* thrombus area 31,451 μm^2, 95% confidence interval [CI] 26,342 – 36,561 μm^2; P = 0.57, ***Figure 6.2***).

6.4.2 VASCULAR VASOMOTOR FUNCTION

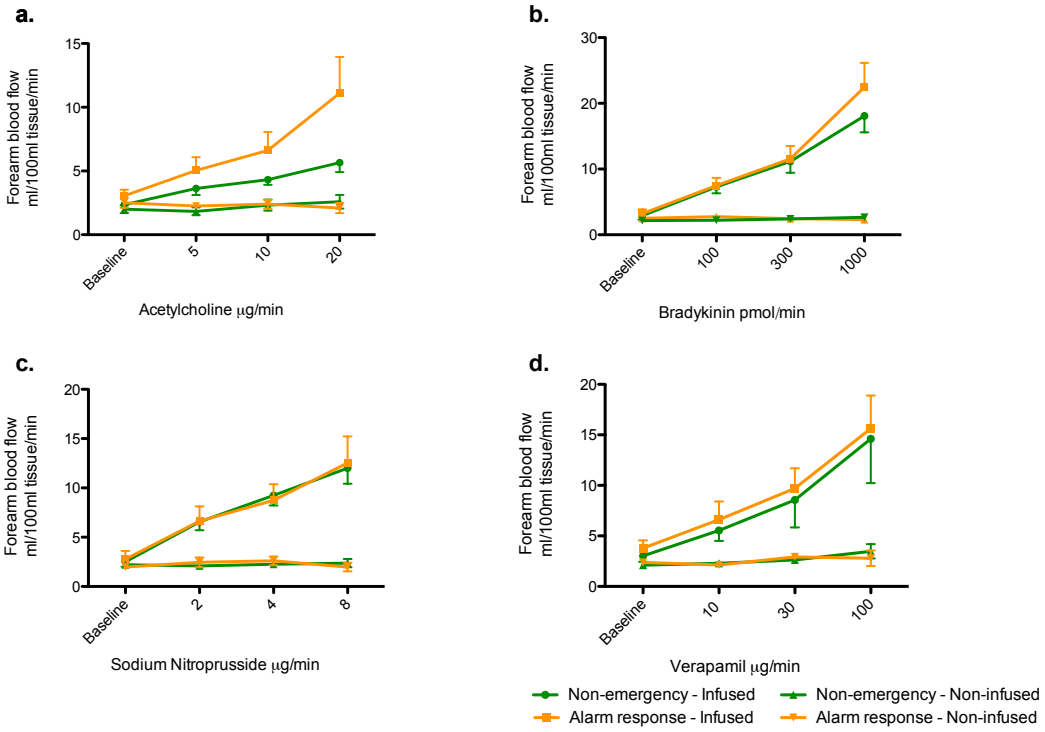
There was a dose-dependent increase in forearm blood flow with each vasodilator (P<0.01 for all). However, there were no differences in blood flow responses to acetylcholine (P=0.11), bradykinin (P=0.54), sodium nitroprusside (P=0.96) or verapamil (P=0.74) following non-emergency activity and alarm response visits ***Figure 6. 3***).

Figure 6-3 Thrombus formation



Thrombus formation under both low and high-sheer conditions in the Badimon chamber were similar following non-emergency duty and alarm response (P>0.05, n=7). The results of the fire suppression studies are shown for information only (n=3). All data expressed as mean ± SEM.

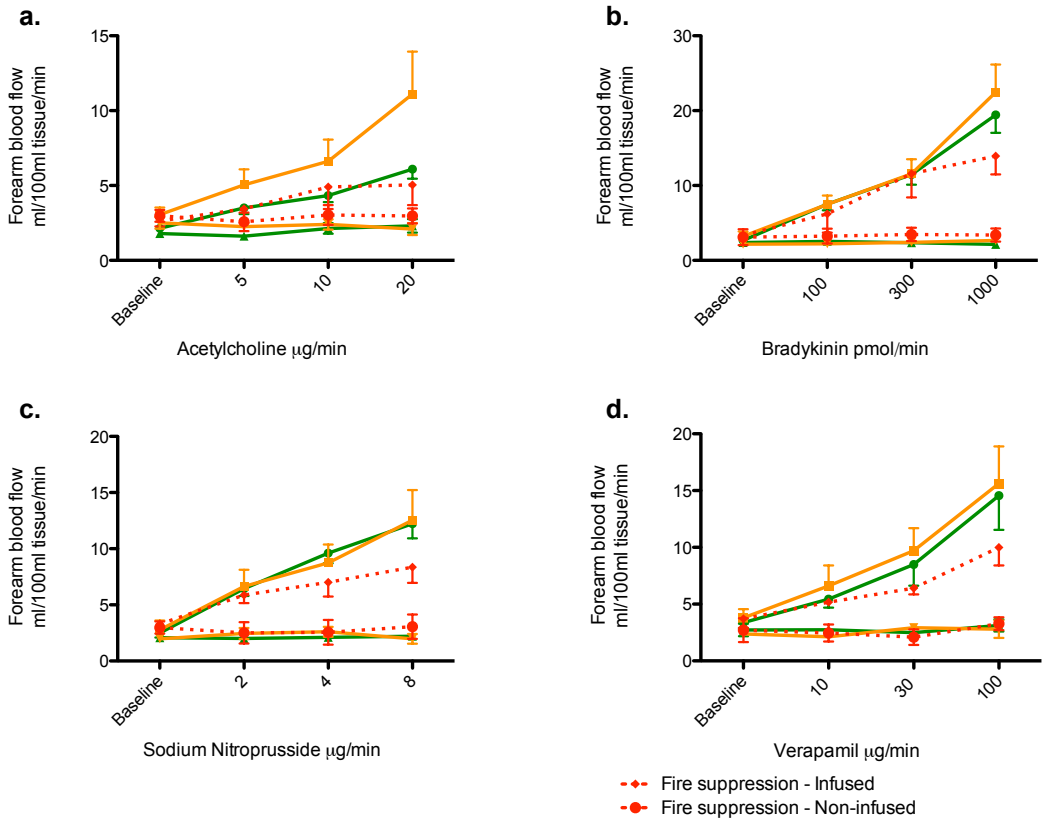
Figure 6-4 Vascular vasomotor function



There was a dose-dependent increase in forearm blood flow with each vasodilator (2-way ANOVA with repeated measures, P<0.01 for all, n=7), however there were no differences in blood flow response to acetylcholine (P=0.11, bradykinin (P=0.54) sodium nitroprusside (P=0.96) or verapamil (P=0.74) between exposures. All data expressed as mean ± SEM.

There are no differences in blood flow in the non-infused arms and therefore these data points are overlaid.

Figure 6-5 Vascular vasomotor function including fire suppression



In addition, the results of the fire suppression studies are shown for information only (n=3). All data expressed as mean ± SEM.

There are no differences in blood flow in the non-infused arms and therefore these data points are overlaid.

6.5 DISCUSSION

Despite our efforts to recruit to this study we were unable to complete enough visits over a two-year period to adequately power the study and are therefore unable to draw firm conclusions from the data presented. We hypothesised that fire suppression would result in impaired vascular function and increased thrombogenicity with non-fire emergency duties resulting in the same but to a lesser extent and that non-emergency duties would result in no impairment of vascular function or increased thrombogenicity. Proving this hypothesis would have given credence to the epidemiological findings of Kales et al and identified the biological mechanisms to support their findings.

The study did not work in its current form for a number of identifiable reasons. Firstly, we were unable to study enough firefighters following a fire suppression exposure. Fortunately, fire suppression in real-life is a relatively infrequent occurrence but for the same reason, fire suppression, where a firefighter had been actively involved in extinguishing a structural fire and had undergone exposure to the relevant occupational risk factors, was rare. Firefighters in this study, as part of routine duties, often attended refuse bin or tenement stairwell fires which consisted of rapid extinguishing of fires with no physical exertion or heat exposure. For this reason, we had to stipulate that a fire suppression exposure had to involve attendance to a major fire suppression operation. Secondly, fire suppression exposures are incredibly

tiring and arduous for those firefighters involved and not surprisingly, if some of our firefighter participants had been up all night actively tackling a fire they did not volunteer themselves to attend for a minimum four-hour research study the following morning, especially if there was a second night duty to undertake that evening. Conversely, all of the firefighters who were randomised to attend for their first study visit following a non-emergency duty completed this visit, mainly as they had managed to sleep overnight without any emergency call outs.

Moreover, the complete lack of control we had over the exposure conditions was another reason this study proved exceedingly difficult to execute. All of the study visits occurred on an ad-hoc basis, only receiving a call to inform that an exposure had taken place in the early hours of the morning for a study to take place that same morning following shift end at 8am. The nature of this study design posed difficulties to firefighters in attending following some exposures if they already had plans for that morning or other responsibilities such as childcare. It was difficult to then make alternative arrangements at such short notice to attend for a study visit.

We explored a number of strategies to attempt to maximise study visits. Firstly, I maintained regular contact with the firefighters involved in the study by intermittent email and phone contact, and station visits. As the study was ongoing for a long period of time it was understandable if the study did not

remain at the forefront of the firefighters' minds following the completion of a night shift. Furthermore, due to the ad-hoc nature of the study, firefighters may have travelled to work the previous night by bike or foot and therefore did not have means to get to the research facility in a timely fashion for a study visit. We therefore informed firefighters that they had the option of travelling to the Clinical Research Facility and back home thereafter by a pre-paid taxi. In the latter stages of the study in a further push to maximise study visits, I negotiated with a few of the research nurses to be 'on-call' at the weekend to open the Clinical Research Facility and help with studies. Prior to this I had only been able to undertake studies Monday – Friday for exposures on Sunday – Thursday night duties when the Clinical Research Facility was open. This enabled me to study firefighters following Friday and Saturday night duty, arguably the busiest nights of the week for firefighter exposures. In addition, I managed to acquire the permission of the Assistant Chief Fire Officer for South East Scotland for the Incident Control Centre, where all fire calls are received and dispatched, to contact and inform myself of any fire suppression activity within the region so I could contact the firefighters participating in the study who may have been involved and ask if they would consider attending for the relevant study visit.

Fire suppression duties represent the culmination of all identified occupational risk factors namely, exposure to extreme heat and strenuous physical exertion, air pollution and psychological stressors. As confirmed by

previous studies, fire suppression when assessed in a controlled fashion by a fire simulation exposure, is associated with increases in heart rate and blood pressure, accompanied by alterations in blood flow, vascular shear stress and electrolyte disturbance; decreased plasma volume, increased blood viscosity and a procoagulatory state (Soteriades et al., 2011, Smith et al., 2016, Kales and Smith, 2017) and in our study low-level myocardial injury and ischaemia (Hunter et al., 2017). However, as demonstrated by Kales et al there is an increased but lesser risk of on-duty cardiovascular events following other firefighter duties such as physical exertion in the form of routine training and responding to non-fire emergencies. Other non-fire emergencies that firefighters routinely and frequently attend are road traffic accidents and search and rescue following natural or man-made disasters. Such events cannot really be simulated in the same way as fire suppression. Although physical exertion is a routine factor in any firefighting duty, the non-fire emergencies also encompass potential psychological stress and air pollution. Both of these occupational risk factors are incredibly difficult to characterise. Psychological stress akin to that of a life-threatening scenario, that firefighters face not infrequently, can only truly be assessed in a real-life scenario.

Air pollution is a heterogeneous and omnipotent risk factor for firefighters, and in a non-fire emergency such as a road traffic accident can the take the form of combustion-derived nanoparticulate, commonly diesel exhaust

particles which we know from previous work to have deleterious cardiovascular effects (Mills et al., 2005, Mills et al., 2007). We have demonstrated that a controlled exposure to wood smoke in concentrations similar to that encountered in a wildland fire did not impair vascular function nor increase thrombogenicity (Hunter et al., 2014). However, a controlled exposure to nanoparticulate and gaseous pollutants encountered in a structural fire is not likely to be possible as this form of air pollution is so diverse and would prove impossible to characterise and reproduce. For that reason, a real-life exposure to fire suppression provides us with the only real opportunity of assessing the cardiovascular effects of this occupational risk factor. Arguably, firefighters should be protected from air pollution by their self-contained breathing apparatus (SCBA), yet, despite this significant smoke exposure occurs during structural fires. Whilst SCBA has improved greatly and is essential when fighting internal structural fires, it does not fully eliminate pollutant exposure, and it is still commonplace to find firefighters extinguishing external fires without any respiratory protection. Moreover, respiratory protection is often abandoned during overhaul (the period immediately after fire suppression), when exposure to fine particulate matter and other toxic chemicals remain very high (Burgess et al, 2001). Interestingly, carboxyhaemoglobin levels were between 3-10% in the small proportion of firefighters whom levels were determined prior to death from coronary heart disease (Kales et al, 2003). Carbon monoxide has also

previously been shown to precipitate myocardial ischaemia at concentrations of >150ppm in patients with coronary heart disease (Allred et al, 1989).

The majority of on-duty cardiovascular events occur in firefighters with recognized traditional cardiovascular risk factors (Kales et al, 2003). The prevalence of cigarette smoking, hypertension, hyperlipidaemia and diabetes mellitus are relatively low in comparison to the general population yet the prevalence of these risk factors amongst the cohort of firefighters who have had fatal cardiovascular events is higher (Geibe et al, 2008). It is likely, therefore, that on-duty cardiovascular events represent a complex interplay between the occupational and traditional risk factors. Moreover, preventing cardiovascular events will no doubt necessitate the modification of both occupational and traditional risk factors. The pathophysiology relating to traditional cardiovascular risk factors has been extensively researched and there are clear evidence-based guidelines and targets for treating the modifiable risk factors.

In this study, we attempted to assess the occupational risk factors by assessing the cardiovascular health of firefighters following exposure to occupational risk factors where they occur in defined firefighting duties. Although we are unable to draw firm conclusions from the data available, we can extrapolate from our previous work (Chapters 3-5) and make some common-sense recommendations. Limiting exposure to occupational risk

factors should be a priority. This could potentially be achieved by increasing the number of firefighters attending any single firefighting duty and therefore sharing the burden of that duty, for example, limiting the number of times a single firefighter has to enter a fire suppression scenario. Ensuring also that respiratory protection is worn at all times, even when active fire suppression duties have ceased or at a busy roadside. Effective hydration should also be routine for all firefighters following any duty and formal cooling specifically following fire suppression

The methodology for this study was carefully considered and scientifically sound but in order to undertake this study again and successfully complete a greater number of study visits we would need to recruit higher numbers of firefighters. Furthermore, a multi-centre approach to this study would likely yield a higher number of real-life fire suppression exposures which would adequately power the study for appropriate analysis of the effects of this particular exposure. There would be logistical difficulties in conducting the studies that would require researchers trained in the methodology we use and equipment available to perform the study in every site involved but these difficulties could be overcome. An alternative would be to undertake some of the less time-intensive studies which could be portable, such as the Badimon Study, within the Fire Station itself following the end of a shift. However, this would be a less comprehensive assessment of cardiovascular function.

6.6 CONCLUSION

To conclude, in this study comparing three distinct and representative periods of firefighter duties we have been unable to complete enough studies to adequately power our analysis and draw firm conclusions about the effect of firefighter duties on cardiovascular health. Further work is required in a real-world setting to more clearly define the occupational risk factors underlying the increased risk of cardiovascular events associated with specific firefighter duties.

CHAPTER 7

FUTURE DIRECTIONS

Chapter 7: CONCLUSIONS AND FUTURE DIRECTION

7.1 SUMMARY OF THESIS FINDINGS

7.1.1 COMPARISON OF VASCULAR FUNCTION AND CARDIOVASCULAR RISK IN EMERGENCY SERVICE PROFESSIONALS

Firefighters are at high-risk of cardiovascular death on-duty compared to other occupations, with coronary heart disease accounting for 45 % of deaths amongst firefighters, 22 % of deaths in police officers and 15 % of all deaths at work. We compare traditional cardiovascular risk factors and vascular endothelial function in firefighters and a matched group of police officers with similar occupational responsibilities.

Firefighters have similar cardiovascular risk profiles to police officers and do not have impairment of vasomotor endothelial function whilst off-duty. We suggest that the excess of cardiovascular events and deaths on-duty in firefighters are due to the acute and transient effects of extreme physical exertion, psychological stress, heat and exposure to air pollutants.

7.1.2 Effect of wood smoke exposure on vascular function and thrombus formation in healthy firefighters

Myocardial infarction is the leading cause of death in firefighters and has been linked with exposure to air pollution and fire suppression duties. Globally, wildland firefighting comprises the single largest role of firefighters. We therefore investigated the effects of wood smoke exposure on vascular vasomotor and fibrinolytic function, and thrombus formation in healthy firefighters.

Wood smoke exposure does not impair vascular vasomotor or fibrinolytic function, or increase thrombus formation in firefighters. Acute cardiovascular events following fire suppression may be precipitated by exposure to other air pollutants or through other mechanisms, such as strenuous physical exertion and dehydration.

7.1.3 Effect of heat and physical exertion on vascular function and thrombus formation in healthy firefighters

Rates of myocardial infarction in firefighters are increased during fire suppression duties, and are likely to reflect a combination of factors including extreme physical exertion and heat exposure. We assessed the effects of simulated fire suppression on measures of cardiovascular health in healthy firefighters.

Exposure to extreme heat and physical exertion during fire suppression activates platelets, increases thrombus formation, impairs vascular function and promotes myocardial ischemia and injury in healthy firefighters. Our findings provide pathogenic mechanisms to explain the association between fire suppression activity and acute myocardial infarction in firefighters.

7.1.4 Effect of fire suppression and emergency duties on vascular function in firefighters

Certain firefighter duties are associated with an increased risk of cardiovascular events. Fire suppression carries the greatest risk of cardiovascular events in on-duty firefighters with non-fire related alarm response and even physical activity related to increased risk of cardiovascular events compared with non-emergency duties. The mechanisms underlying increased risk are likely to be defined by the exposure to the constellation of occupational risk factors that these duties entail. By comparing cardiovascular health in firefighters following three distinct periods of duty we aim to further delineate the cardiovascular risk posed by occupational risk factors.

In this study comparing three distinct and representative periods of firefighter duty we were unable to complete enough studies to adequately power an analysis and draw any firm conclusions about the effect of these duties on cardiovascular health. Further work is required in a real-world setting to more clearly define the occupational risk factors underlying the increased risk of cardiovascular events associated with specific firefighter duties.

7.2 Future Directions: Interventions to mitigate risk

7.2.1 Monitoring Equipment

Given the hostile environment that firefighters operate in, it has proved exceedingly difficult to undertake accurate real-time monitoring of indices such as heart rate, which serves as an indicator of physical exertion, cardiac strain and respiratory rate, and is an important determinant of how fast an individual will consume air. As assessment of fitness moves away from traditional cardiopulmonary fitness testing and towards a more job-specific approach to fitness assessment we will require advanced monitoring with equipment that can withstand the unique occupational conditions and provide reliable and reproducible data. A number of biometric vests are now available that are able to measure various physiological parameters in real-time. One of which has been assessed specifically in simulated firefighting scenarios and has been shown to be accurate in this setting when measuring heart rate but further research is required to improve on this accuracy of respiratory rate measurements in certain activities (Smith et al., 2014) . Such technology may also be utilised in real-life firefighting scenarios and provide the Fire Service with more objective and important safety monitoring especially during large scale firefighting operations where firefighters can be in attendance for many hours, if not days, such as wildland fires. Securing investment for this technology and the manpower required for the active

monitoring of results will however prove a major drawback to its widespread implementation.

7.2.2 ACTIVE COOLING AND TARGETED REHYDRATION

We have previously demonstrated the adverse vascular and prothrombotic effects of extreme heat and physical exertion. Impairment of firefighters' cognitive function has been demonstrated by other groups in similar settings (Smith and Petruzzello, 1998, Watt et al., 2016). Correcting the sequelae of fire suppression such as dehydration or the increase in core body temperature in an attempt to restore normal physiology with cooling and targeted rehydration could potentially reverse such changes and moreover, are inexpensive and without risk of harm.

Forearm and hand cooling appears to be more effective than other active cooling techniques such as use of a misting fan, cooling vest or passive cooling (McLellan and Selkirk, 2006, Carter et al., 1999). Forearm immersion in cold water extended time to exhaustion by 60% and prevented excessive rise in core body temperature compared to passive cooling in firefighters wearing full PPE and SCBA who had repeated exposures to heat and exercise separated by brief rehabilitation periods (McLellan and Selkirk, 2006). However, during active firefighting operations, it is not always practical to cool via these methods as it would require removal of clothing

and PPE which is not often feasible. A recent study assessing contemporary cooling vests and comparing them to a cooling gel found that the vest was not only practical but was more effective in attenuating physiological responses and maintaining cognitive function (Hemmatjo et al., 2017).

Active cooling was shown by Burgess and colleagues to reduce core body temperature, heart rate (around 20bpm) and leucocytosis (Burgess et al., 2012) when employed with a cooling chair. The effect on all of these variables was unsurprisingly more marked with a longer duration of treatment. There was no effect on systolic blood pressure. Importantly, active cooling appears to be well tolerated and there is some anecdotal evidence that subjects find this more comfortable than passive cooling, and it also appears to stop sweating earlier. By contrast, active cooling has not been shown to reverse many of the prothrombotic effects measured with simulated fire simulation.

The detrimental vascular and thrombotic effects that we have demonstrated in response to fire simulation exposure (*Chapter 5)* have important implications for firefighters in a real-life fire suppression scenario when firefighters potentially have multiple entries into the same fire. Limiting duration of the exposure would be a simple and sensible approach to this problem, however with overall reductions in numbers of operational firefighters across the UK, this is not likely to be a feasible or realistic solution

long-term. Furthermore, large-scale operations such as the recent Grenfell Tower fire, although thankfully exceedingly rare, necessitate all available operational personnel to work almost continuously to save and preserve life with often a disregard for own personal safety or protocol.

All firefighters are encouraged to cool actively and rehydrate during and after fire suppression but the responsibility lies with the individual firefighter to do so. Often during large scale operations, firefighters are committed multiple times to active fire suppression with little time for rest in between other than to switch air cylinders. They are provided with bottles of water but often will only take a couple of mouthfuls to quench their thirst rather than ingest the appropriate amount of fluid required for rehydration and to attain euvolaemia. We demonstrated that fluid losses of ~500g occurred within a 20min training exercise and would hypothesise that the fluid losses would be much more marked with multiple entries into a real-life fire suppression. We demonstrated, as others have done, that firefighters are not so aware of the physiological strains that their bodies are undergoing and concluded that by measuring body weight pre- and post-fire suppression, firefighters would have an appropriate target for rehydration. Rehydration and cooling need to be made mandatory and monitored by firefighters managing operations. Moreover, education of the importance of these simple interventions to firefighters at operational level will be the key to change in this instance. Future research should be focused on evaluating and determining the

magnitude of these simple interventions.

7.2.3 ANTI-THROMBOTIC AGENTS

On the basis of previous studies revealing that fire simulation exposure led to a prothrombotic state with increased platelet aggregability and increases in thrombotic and fibrinolytic potential (Smith et al., 2011, Smith et al., 2014, Burgess et al., 2012), an interventional trial of aspirin in firefighters was undertaken despite the lack of robust mechanistic data supporting this as an intervention (Hostler et al., 2014). The double-blind, placebo-controlled study where firefighters were given aspirin or placebo before and/or after walking on a treadmill in heat and in full PPE revealed that platelet activation was blunted by daily aspirin and also within 60min of a post exercise single dose of aspirin. Platelet activation was measured by platelet closure time. Whilst we do agree with the rationale that interventions such as providing firefighters with aspirin could be used to reduce cardiovascular risk, this hypothesis would need to be prospectively tested in simulated or real-life firefighting conditions, given that there is potential for harm resulting from the unintended effects of the treatments. Indeed, Smith et al (Smith et al., 2016) recently demonstrated in a small placebo-controlled study that neither the short- nor long-term aspirin pre-treatment in firefighters prevented the adverse effects of fire simulation on platelet function, coagulation, or fibrinolysis. Although aspirin clearly reduces cardiovascular risk in

susceptible individuals, larger trials would be required to determine whether aspirin is beneficial or harmful to firefighters as a whole. Future research should evaluate simple interventions such as cooling and rehydration before seeking a pharmacological approach to reducing cardiovascular risk. Ultimately any strategy will need to be evaluated in a real-life fire suppression setting.

7.2.4 Modifiable cardiovascular risk factor reduction and health promotion

The health and wellbeing of firefighters is not only a concern for the Fire Service but as it impacts directly on the safety of the public, their risk of cardiovascular disease should be of national concern. There are modifiable cardiovascular risk factors that can be identified and addressed and all efforts should be made within Fire Service occupational health departments to do so. Within the Scottish Fire and Rescue Service there have been efforts made towards cardiovascular risk factor identification with the introduction of point-of-care total cholesterol monitoring. The point-of-care devices have been shown to demonstrate reasonable accuracy when compared to laboratory venous blood testing with correlation coefficients for total cholesterol of 0.86 (coefficient of variation (CV) 12%) (Plüddemann et al., 2012). However, at levels near decision thresholds of diagnosis and treatment, the machines overestimate triglycerides and HDL, and

underestimate LDL. Cholesterol testing within the Fire Service was not made mandatory and was offered to firefighters with a number of them declining the test. Furthermore, there was no clear pathway in place for dealing with firefighters where hypercholesterolaemia had been identified. It was simply communicated to them and the onus was then with them to contact their own GP for further assessment.

Whilst this move towards recognizing the importance of and identifying risk factors amongst firefighters should be commended, cardiovascular risk factors should be assessed in a holistic fashion by an appropriately trained professional that can counsel firefighters who have identified risk factors, undertake a cardiovascular risk calculation, provide lifestyle advice and commence and monitor appropriate treatment. There is a strong argument for Fire Service occupational health physicians and nursing staff to be trained in this regard and they could therefore take ownership of cardiovascular risk management especially as they understand the occupational demands placed on firefighters unlike General Practitioners.

Obesity is a global epidemic similarly effecting firefighters. As aforesaid, a number of studies have worryingly reported that over 70% of firefighters are overweight or obese (Geibe et al., 2008). Unlike other cardiovascular risk factors, the presence of obesity can have a very demonstrable impact on whether a firefighter is able to carry out their job safely which is only usually

evidenced in an inability to meet mandatory fitness standards. Currently it appears to be at this stage when interventions are instigated in the form of personalized fitness programs and dietary advice regarding weight loss. A case in one of the previous constituent Fire Services within the Scottish Fire and Rescue Service saw a firefighter who was grossly obese eventually lost his job in 2009 when he failed to lose weight or achieve the minimal safe fitness standard (http://www.telegraph.co.uk/news/uknews/scotland/4247714/Fat-fireman-sacked-for-failng-to-slim-down.html). There was an outcry when he was given a three-month period to lose in excess of 3 stone which was felt amongst nutritional and fitness experts at the time to be a completely unrealistic target in the allotted time frame. The firefighter in question was over 20 stone at the time of dismissal. If he had been determined to be overweight or obese previously while he was still able to meet the fitness requirements then interventions at this stage may have averted the need for dismissal. At present, UK firefighters, if maintaining physical fitness standards, can go without contact with the occupational health department for up to three years. In the US, there is often no contact with occupational health and incumbent firefighters can often have no further fitness assessment following recruitment. Arguably, if contact with occupational health departments were maintained on at least an annual basis then weight could be monitored and identified as an issue requiring attention. Intervention earlier in a firefighter's career could avert early dismissal or retirement. The

threat of dismissal or of mandatory retirement, while it may be construed as harsh, ultimately is to protect the firefighter and the community as a whole.

Appropriate education amongst firefighters is key to achieving cardiovascular risk factor reduction. Promotion of exercise and healthy diet within the Fire Service should be commonplace. Additionally, as adequate levels of cardiopulmonary fitness are a prerequisite for occupational safety there should be allocated time within a firefighter's working day for maintenance of fitness. In a previous study it was determined that 97% of firefighters wanted their employer to offer health education with the topics of interest being nutrition, fitness and stress management and the desired means of communication being written and spoken in the form of presentations (Kay et al., 2001). Furthermore, 75% of firefighters specifically wanted to know more about healthy eating (Yang et al., 2015). Communicating risk to patients is something we struggle with as doctors especially for primary prevention of cardiovascular disease where most risk factors are asymptomatic. Developing an understanding of lifetime risk of cardiovascular disease and providing comparative information to patients; for example actual heart age *versus* chronological age or predicted age at first cardiovascular event. This would provide a more tangible concept for patients to grasp and can help them understand that the risk, although invisible, is very real. Additionally, enforcing bans on smoking within worldwide Fire Service buildings is strongly

advised. Although in Scotland, smoking in a public space is illegal, smoking bans should be extended to include the fire ground.

7.3 Future Directions: Potential Studies

7.3.1 Establishing a national database

Epidemiological research in Scotland in eminently more straightforward as all residents have a unique identifying number that links directly to databases where information about previous coded diagnoses, current medication and mode of death are prospectively recorded. The amalgamation of all constituent Fire and Rescue Services into the Scottish Fire and Rescue Service back in 2014 should further ease the ability of occupational health research within this Service in particular. The desire of the Fire Service to embark on such research is mixed. Throughout the course of my research I have endeavored to engage firefighters, both at operational and management level, and occupational health personnel in order to develop an understanding that whilst the numbers of firefighters who have cardiovascular events on-duty in the UK are potentially few they are nonetheless important and arguably avoidable. In my opinion, there remains a reticence, at least amongst management level firefighters, to investigate adequately the prevalence and incidence of cardiovascular disease in firefighters. This reticence is in part based on an unfounded yet common belief amongst UK firefighters that US firefighters are fundamentally different both in their approach to safety as firefighters and to their health and fitness. As the wealth of epidemiological data regarding cardiovascular events in

firefighters is derived from the US firefighter population this further biases this belief.

There remains much to be done in gaining an understanding of the prevalence of cardiovascular risk factors within the UK Fire Service and appropriately identifying those that may be most at risk of cardiovascular events. I have demonstrated during this research that even in healthy UK firefighters, with no cardiovascular risk factors, fire simulation causes dysfunction of the biological mechanisms underlying myocardial infarction. By disseminating the research findings, I have worked to dispel these beliefs and fostered an understanding that all firefighters may be at risk. I believe I have therefore, laid the ground for future research to be undertaken and on a national platform at meetings have advocated for collaborative research within Scotland and the UK and for data sharing within a national database. Establishing a national database in the UK should be a priority. I envisage that all occupational health departments within individual Fire Services could input data regarding risk factors and history of previous cardiovascular events. It has already been recommended that fire brigades should capture, collate and analyse information regarding all sickness absence and ill-health retirements with the Home Office asked to provide protocols for this (Home Office, 2000). This would therefore provide us with up to date information about the incidence and prevalence of risk factors and cardiovascular events

in UK firefighters and allow targeted interventions as above that could be undertaken in order of risk.

Another area requiring standardisation is the categorisation of on-duty firefighter deaths in the UK. In the US, a firefighter death is categorised as an on-duty death if it occurs on duty or within the following 24-h period. This reflects evidence that there is a lag in cardiovascular events amongst firefighters following exposures and ensures families are appropriately compensated in the unfortunate event. In the UK, a firefighter death is only classified as being on-duty if it occurs at work. It would make sense for the UK Fire Service to bring categorisation of on-duty death in line with the US where such guidance already exists.

7.3.2 Investigating if firefighters have increased lifetime risk of cardiovascular events

During this period of research, I spoke with many firefighters who recalled anecdotes of retired colleagues who had suffered cardiovascular events shortly after retirement at a relatively young age. There is no current evidence to support an association between firefighters and increased lifetime risk of cardiovascular events. However, a group of currently employed US firefighters were found to have significantly greater coronary atherosclerosis and calcium on CT than matched controls. Interestingly,

these firefighters had significantly more lesions in the left main and left anterior descending arteries (Pillutla et al., 2012). It seems highly plausible that over a career spanning 25-30 years, with multiple potential vascular insults secondary to occupational exposures, that retired firefighters may have a greater atherosclerotic burden than non-firefighters. I would envisage a study to further test this hypothesis having two parts: a retrospective cohort study to include all currently retired firefighters within the UK where we record prevalence of cardiovascular risk factors and cardiovascular events and compare this with a matched control population. The second part would be a prospective case-control study where we recruited all firefighters as they retire. We would assess risk factors and undertake a CTCA to assess for asymptomatic coronary artery disease and then compare the atherosclerotic disease burden with a group of non-firefighter matched controls. This would allow us to address this research question and potentially identify firefighters with asymptomatic coronary artery disease and intervene with preventive therapies. This intervention in patients with non-obstructive coronary artery disease on CTCA has been shown to half rates of fatal and non-fatal MI in patients presenting with chest pain to an outpatient setting (Williams et al., 2016).

7.3.3 COMPARISON OF FIRE SIMULATION EXPOSURE WITH EXERCISE ALONE

In peer-review of the fire simulation exposure study (Chapter 5), we were asked if we had considered if our findings of impaired vascular function, increased thrombotic potential and myocardial injury could be secondary to either intense physical exertion or heat exposure in isolation. We considered during the design of our study and it was raised by our funders, the British Heart Foundation, at this stage. However, we wished to simulate the effects of real fire suppression activity in our study as closely as possible. Firefighters are never exposed to heat without exercise, and therefore whilst it would be interesting to evaluate these components separately, neither are avoidable for firefighters and this was not considered a priority by the occupational physicians or firefighters contributing to our research group.

For the purposes of the peer review, we have evaluated forearm vasomotor function and high sensitivity troponin I concentrations after intermittent exercise at moderate intensity (minute ventilation of 20-25 L/kg/m^2) for 1 h in a contemporaneous age-matched control group of healthy volunteers (Hunter et al., 2014, Mills et al., 2011). Vasodilatation in response to acetylcholine (ACH) and sodium nitroprusside (SNP) was attenuated following fire simulation exposure compared to this moderate intensity exercise alone ($P<0.0001$ for ACH, $P=0.003$ for SNP). Similarly, there was no effect of exercise alone at least at this intensity on cardiac troponin I concentrations at

1 h or 24 h (P=0.5). Whilst the intensity of exercise clearly differs between these study populations, these observations suggest that the effects of fire suppression on cardiovascular health are not exclusively a consequence of intense exercise. A further study assessing the same fire simulation exposure (*Chapter 5*) with a control exposure of an equivalent period of physical exercise in the absence of fire simulation using identical methodology would be required and would be useful in determining the exact mechanism of myocardial injury, vascular dysfunction and thrombosis. An additional control exposure of heat exposure alone, would be of interest but not helpful for progressing our understanding of underlying mechanisms of cardiovascular events in firefighters as they are never exposed to heat without physical exertion.

7.3.4 Real world mechanistic study

In order to further understand the effects of real-life fire suppression duties on cardiovascular health we designed a study (*Chapter 6*) which was incredibly challenging to undertake within, what was at the time, a district fire service. The main limiting factor was the number of real-life fire suppression exposures. Fortunately, there are very few large-scale fires in this country but it was a very real impediment to this ambitious research study. Whilst I do believe our methodology was sound, the fact that I was unable to undertake more studies with the number of firefighters recruited suggests we may need to recruit higher numbers of firefighters. Also, a multi-centre approach to this

study would likely yield a higher number of real-life fire suppression exposures which would adequately power the study for appropriate analysis of the effects of this particular exposure. There would be logistical difficulties in conducting the studies that would require researchers trained in the methodology we use and equipment available to perform the study in every site involved but these difficulties could be overcome.

7.3.5 INTERVENTIONAL STUDIES

Ideally any intervention applied in an attempt to mitigate occupational risk should be trialed within real-life firefighting operations. Considering that it was so difficult to undertake a study to assess mechanisms underlying increased risk of cardiovascular events in this setting and with the knowledge of the unpredictability and time-pressures of real-life emergency situations, it would be incredibly ambitious to attempt any real-life interventional study that involved asking firefighters to undertake any intervention that might be seen to detract from the job at hand. Furthermore, we would be unlikely to get approval at management level. However, fire simulation exposures are part of routine and ongoing firefighter training and would be an appropriate starting point for an interventional study. I would envisage undertaking an interventional study with an open label case crossover design using same methodology as used in our fire simulation exposure (*Chapter 5*) but on this occasion, have two visits for fire simulation exposures and on one occasion

randomize firefighters to receive both active cooling and targeted rehydration to assess if this mitigates the adverse vascular and thrombotic effects and limits myocardial injury as was demonstrated in this study (Chapter 5). Interventional studies assessing pharmacological interventions such as aspirin or beta blockers, as was suggested in a letter to Circulation journal about our work (Hunter and Mills, 2017), could be undertaken on a small scale as a pilot study to investigate if they mitigate the adverse effects of fire simulation exposure, however we would need to undertake a large scale randomized control trial to assess the overall safety and effectiveness of such pharmacological interventions.

7.3.6 Occupational risks extrapolated to other occupations or general population

Whilst not relevant to firefighters, we have been contacted during the period of this research by workers in other occupations and asked by journalists and colleagues alike if our research findings can be extrapolated to other occupational groups such as workers in power stations who are subject to high ambient temperatures. Firefighters are a unique group that are exposed to many occupational risks on-duty often simultaneously. However, some of these occupational risk factors such as heavy physical exertion, high ambient temperatures and air pollution may be encountered by other occupational groups or by members of the general population, although usually in

isolation. The findings in this study are therefore not directly applicable to the other occupational groups or the public at large. Occupation specific research should be conducted in order to inform individual occupational groups on risks pertaining to them whilst ‘at work’. However, if we were to generalize any of our observations then we would recommend anyone who is exerting themselves in high ambient temperatures should keep well hydrated and allow time to cool down afterwards.

7.4 CLINICAL PERSPECTIVE: PROTECTING FIREFIGHTERS IN FUTURE

Despite major advances in cardiovascular medicine, over the last two decades the morbidity and mortality from on-duty cardiovascular events amongst firefighters has not significantly changed (Kales et al., 2007). Whilst we are unable to significantly reduce or impact upon firefighters' occupational risk factors we can certainly modify and improve their underlying cardiovascular risk profile.

7.4.1 FORMAL RISK STRATIFICATION FOR ALL FIREFIGHTERS

Most on-duty cardiovascular events are likely to be work precipitated but crucially appear to be concentrated amongst the most susceptible individuals. It has been demonstrated repeatedly that on-duty cardiovascular events occur primarily in firefighters with underlying disease (known or previously subclinical) or those with excess cardiovascular risk factors (Holder et al., 2006, Kales et al., 2003, Geibe et al., 2008, Glueck et al., 1996). Efforts, therefore, should be concentrated primarily on formally risk stratifying firefighters which should be mandatory for all and comprise part of a routine health and wellbeing assessment ideally on an at least an annual basis.

The national database that I suggest should be established could be set up to incorporate information for occupational health physicians about

recognised cardiovascular risk factors, the appropriate diagnostic thresholds and target treatment levels for modifiable risk factors such as blood pressure and cholesterol, and also state referral criteria for specialist care if required.

7.4.2 Computed tomography coronary angiograms for firefighters above 40

Increasing demands are being made of an aging fire service with the age of retirement in the UK now set at 60 years of age. It may, therefore, become appropriate and necessary for the fire service to investigate for the presence of underlying coronary disease in firefighters above the age of 40 or below if constellation of risk factors suggest the risk of coronary event is high, with CTCA. It is well established that the majority of coronary events occur in arteries that have non-obstructive coronary plaque and in firefighters especially it is likely that plaque rupture likely accounts for most cardiovascular events rather than obstructive disease which is likely to be symptomatic. Amongst a group of asymptomatic firefighters undergoing coronary calcium scoring, 87% had a higher than average Agatston score compared with a national database and a score above the 75th centile was found in 57 % of firefighters (Santora et al., 2013). Coronary calcium was only identified in men older than 34 years. Interestingly there was no correlation between traditional cardiovascular risk factors and the presence of coronary calcium, and despite a similar distribution of risk factors to the

general population, firefighters have higher than average amounts of coronary calcium. Therefore, use of cardiac CT in firefighters may provide a more accurate method of risk assessment in this population. Furthermore, improved cardiovascular outcomes have now been reported following on CTCA (Williams et al., 2016) Amongst firefighters, a strategy of anatomic screening has also been found to lead to significant cost savings (Budoff et al., 2009).

The use of CTCA is becoming more mainstream and indeed has become one of the first line investigations in patients with stable chest pain. Contemporary CTCA is safe, can be undertaken in anyone and owing to current technology can be performed with a relatively low radiation dose, similar to if not less than that of a coronary calcium scan with the benefit of gaining important anatomical data. If firefighters are to be put continually into situations that may provoke coronary events in those with underlying unknown asymptomatic coronary disease then arguably it is better to be forewarned and for them to either be removed from active front line duties or at the very least to have restrictions placed upon the amount of work/exposure they are able to undertake. This may not be a popular strategy amongst firefighters but ultimately there is a cause and effect relationship between firefighting and acute coronary events and it could be construed as careless or even negligent to let such firefighters continue to work unchecked. In the US there exists “Heart Presumption” legislation that

firefighters who die from or suffer a coronary event on duty are entitled to government-funded compensation for the same. This begs the question as to why other countries do not follow the example of the US in this regard. It may have the effect of focussing our attention and efforts into addressing risk factors in this group. Conversely, it does not appear to have had this effect in the US where numbers of those suffering from coronary events or dying from the same have remained relatively static. The United States is unique in that the proportion of volunteer firefighters to career firefighters is high (72% to 28%) (Smith et al., 2013) and the fitness and health standards are not routinely assessed and unlikely to be met.

7.4.3 PROMOTION OF FITNESS AND HEALTH WITHIN WORKING ENVIRONMENT

Many American firefighters receive medical and physical fitness testing at the beginning of their professional career i.e. recruitment and no subsequent formal reassessment over a 20-30 year work span but yet are expected to perform the same job as younger and fitter firefighters (Soteriades et al., 2011). In Scotland, routine medical and fitness testing occurs every three years or more frequently if fitness levels are substandard.

There is still no national policy or official accepted guidance regarding the implementation of minimum physical fitness standards or maintenance of physical fitness thereafter in the UK. The expert recommended minimum

level of for cardiopulmonary fitness is a $VO_{2\ Max}$ of 42 mL/kg/min (Stevenson, 2009). A recent study confirmed that firefighters with an aerobic capacity below an occupational fitness standard of 42.3 mL/kg/min would not be guaranteed to be safe and effective in their ability to complete necessary roles within their occupation. And further indicates that a lower $VO_{2\ Max}$ standard of 35 ml/kg/min, which the majority of Fire Services in the UK employ for continuation of work with remedial training amongst operational firefighters, is potentially unsafe for the majority of firefighters (Siddall, 2014).

Although a $VO_{2\ Max}$ of 42 mL/kg/min is accepted as the average fitness level in 30-39 year old males (***Table 1.1,*** *Chapter 1*), for a 60 year old male this is between the 80th and 90th centiles for age. For females, only 2 5% will meet the fitness criteria to become firefighters and owing to the gender differences in fitness it is likely a substantially larger proportion of women will find it hard to maintain fitness levels to continue to work until the age of 60. There is a call amongst experts in the field of firefighter fitness that a fitness standard must reflect the occupational demands of the role and suggest a more task specific measure of fitness assessment which is relevant to the job rather than using a treadmill or bicycle ergometer to measure cardiopulmonary fitness. Some Fire Services have already developed specific training programmes that focus on the actual physical demands of the firefighting role. Although firefighters have a professional responsibility to maintain fitness, programmes for fitness and health promotion which are integrated

into a working day for firefighters and not expected over and above working hours are likely to have a higher uptake and be more achievable. If firefighters are being asked to work into their sixties and a desired fitness level is a prerequisite for the job, there is a duty of care of the Fire Service to provide time within working hours to achieve the fitness standard which becomes less attainable with age. Various Fire Services have sought to introduce health and fitness promotion by employing ‘fitness managers’ who are generally sports scientists to undertake fitness testing and administer appropriate advice and training programmes to improve fitness levels. In addition, they work closely with the occupational health team. This is a move towards improvement although this service should be available to all to maintain long-term fitness levels rather than reactionary following identification of substandard fitness.

The Department for Communities and Local Government, the Home Office and the National Joint Council for Local Authority Fire and Rescue Service collaborated to publish a best practice guide for firefighter fitness in 2016 (Firefighter Fitness Joint Working Group, 2016). A number of recommendations are made but the responsibility remains with the individual Fire Services to implement such recommendations. They advocate for more frequent fitness checks as the state of physical fitness can decline over a short period of time, any problems with attaining fitness can be identified and intervened upon at an earlier stage. Routine six-monthly fitness checks are

therefore considered to be best practice for all operational personnel. In addition, Fire Services, despite cost constraints, should also be supporting firefighters to maintain fitness by providing adequate and appropriate fitness training equipment.

With the recognised role of increased cardiopulmonary fitness in offsetting cardiovascular events, focussing efforts on improving overall fitness within the Fire Service will go a long way to ensure improved safety for firefighters in the future.

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APPENDIX: Publications Arising from Thesis

1. Hunter A, Mills N, Newby D. Combustion-derived air pollution and cardiovascular disease. *Br J Hosp Medicine Lond Engl 2005*. 2012; 73:492–497.
2. Hunter AL, Unosson J, Bosson JA, Langrish JP, Pourazar J, Raftis JB, Miller MR, Lucking AJ, Boman C, Nyström R, Donaldson K, Flapan AD, Shah AS, Pung L, Sadiktsis I, Masala S, Westerholm R, Sandström T, Blomberg A, Newby DE, Mills NL. Effect of wood smoke exposure on vascular function and thrombus formation in healthy fire fighters. *Part Fibre Toxicol*. 2014; 11:62
3. Hunter AL, Shah AS, Langrish JP, Raftis JB, Lucking AJ, Brittan M, Venkatasubramanian S, Stables CL, Stelzle D, Marshall J, Graveling R, Flapan AD, Newby DE, Mills NL. Fire Simulation and Cardiovascular Health in Firefighters. *Circulation*. 2017; 135:1284–1295.
4. Hunter AL, Mills NL. Response by Hunter and Mills to Letters Regarding Article, “Fire Simulation and Cardiovascular Health in Firefighters”. *Circulation*. 2017; 136:976–977.